AUS DEM LEHRSTUHL FÜR
KLINISCHE CHEMIE UND LABORATORIUMSMEDIZIN
PROF. DR. GERD SCHMITZ
DER FAKULTÄT FÜR MEDIZIN
DER UNIVERSITÄT REGENSBURG

POSTPRANDIAL LIPIDOMIC RESPONSE IN DIABESITY AND THE METABOLIC SYNDROME

Inaugural – Dissertation
zur Erlangung des Doktorgrades
der Medizin

der
Fakultät für Medizin
der Universität Regensburg

vorgelegt von
Afssun Kaviany
aus Gießen

2014

AUS DEM LEHRSTUHL FÜR
KLINISCHE CHEMIE UND LABORATORIUMSMEDIZIN
PROF. DR. GERD SCHMITZ
DER FAKULTÄT FÜR MEDIZIN
DER UNIVERSITÄT REGENSBURG

POSTPRANDIAL LIPIDOMIC RESPONSE IN DIABESITY AND THE METABOLIC SYNDROME

Inaugural – Dissertation
zur Erlangung des Doktorgrades
der Medizin

der
Fakultät für Medizin
der Universität Regensburg

vorgelegt von
Afssun Kaviany
aus Gießen

2014

Dekan:	Prof. Dr. Dr. Torsten E. Reichert
1. Berichterstatter:	Prof. Dr. Gerd Schmitz
2. Berichterstatter:	PD Dr. Marcus Fischer
Tag der mündlichen Prüfung:	16.Juli 2015

Table of Contents

1 Introduction

“Diabetes and cardiovascular disease (CVD) are, together with cancer and chronic respiratory disease, the world’s biggest killers, causing an estimated 35 million deaths each year, 80% of which are in low- and middle-income countries.” [1].

In 2008, the WHO estimated that more than 10% of the world’s adults – 1.4 billion adults in total – were overweight. The incidence of overweight is age-dependent and increases between the ages of 20-50 and decreases between the ages of 60 and 70 [2]. The WHO published an estimate for 2010, according to which 77.2% of men in Germany over the age of 30 had a BMI higher than 25. Of the 1.4 billion adults, over 200 million men were obese [2]. Obesity is a spreading disease, which is not only a serious problem in high-income countries, but also a rapidly growing problem in developing countries. The prevalence of obesity is progressing worldwide [2].

The graphic of the international association for the study of obesity in Figure 1 shows the prevalence of obesity on the different continents.

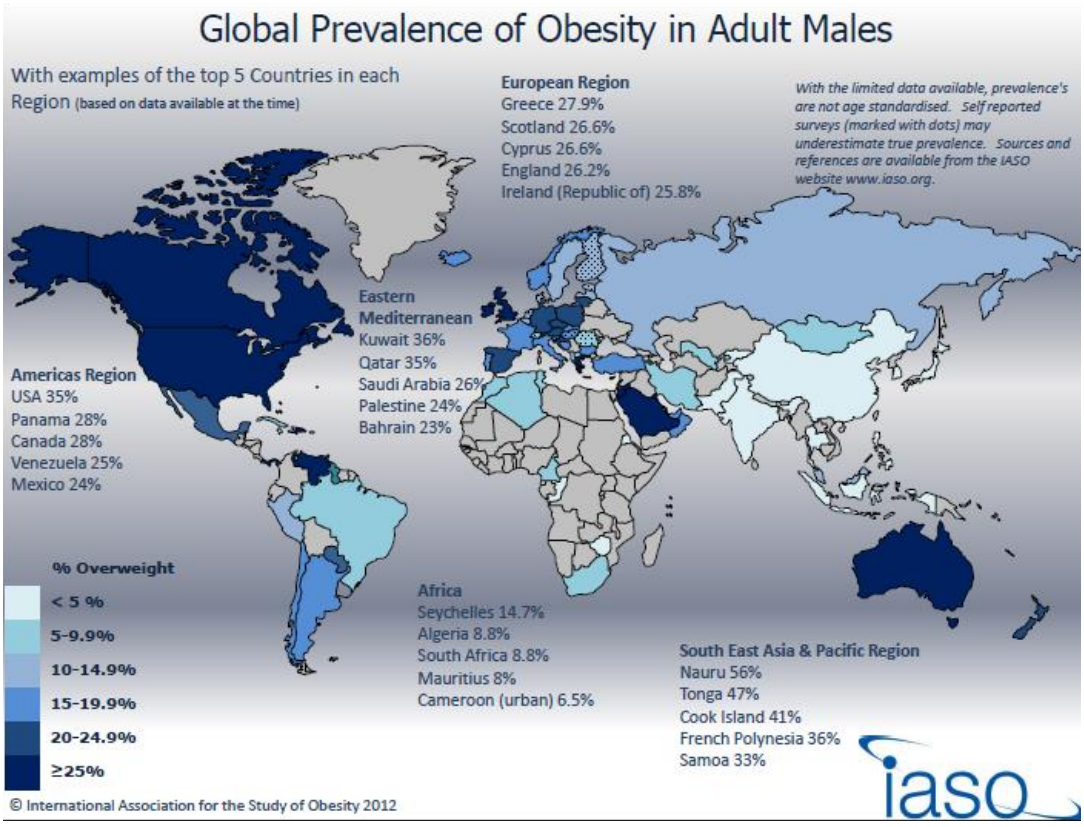


Figure 1: Prevalence of **obese men in the world**, published by the international association for the study of obesity in 2012 (1).

Worldwide, at least 2.8 million adults die each year as a result of being overweight or obese [2]. In addition, 44% of the diabetes burden, 23% of the ischemic heart disease burden and between 7% and 41% of certain cancer burdens are attributable to overweight and obesity [2]. The following graphic by the DAG (Deutsche Adipositas Gesellschaft) (Figure 2) represents the weight distribution (%) of the German population in the years 2005/06. It shows that in total, more than half of the German population (58.2%) suffered from overweight and obesity. 20.8% were obese and 37.4% were overweight, but there are only 40.4% of the population with normal weight. In summary, it can be said that overweight is also a significant problem in Germany.

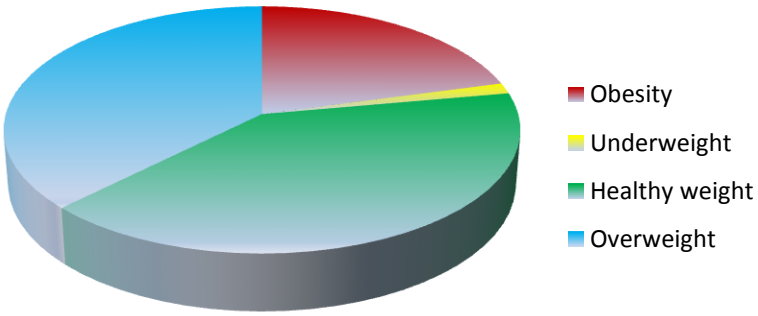


Figure 2: **Deutsche Adipositas Gesellschaft** (DAG) representation of the percentage of obese, overweight, healthy weight and underweight people in Germany (2).

The metabolic syndrome, classified by ATP (Adult Treatment Panel) III criteria, in Germany in 2007 showed a prevalence of 18% in women and 25% in men [3]. The risk of a cardiovascular event in subjects with metabolic syndrome is about three times higher than in the normal population [3]. 60% of all subjects with hypertension have the metabolic syndrome [4].

The metabolic syndrome represents a complex medical condition which is associated with an increased risk for the development of diabetes, chronic low-grade inflammation of the vessel wall, stroke and cardiovascular disease. Interest in the metabolic syndrome has been increasing since diseases connected to it are major health problems in industrialized countries. Early detection and individualized risk stratification are key factors in lowering disease-

related morbidity and mortality connected to obesity, type 2 diabetes and the metabolic syndrome.

1.1 Definition of the metabolic syndrome and obesity

1.1.1 Obesity

The DAG (Deutsche Adipositas Gesellschaft) and WHO (World health organization) define obesity as more than normal or excessive accumulation of fat tissue in the body, which results in too much weight and presents risks to health [2], [5]. One way to measure obesity is the body mass index (BMI), i.e. a person's weight (in kilograms) divided by the square of his or her height in meters. A person with a BMI equal to or more than 25 is considered overweight [2]. A person with a BMI of 30 or more is generally considered obese[2] .

The WHO classifies obesity into three groups [6]:

I: BMI: 30-34.9
II: BMI: 35-39.9
III: BMI: > or = 40

Since obesity-associated diseases correlate with abdominal fat, the waist circumference or the waist-to-hip (W/H) ratio is more accurate than the BMI, because the BMI does not distinguish between central and peripheral obesity [6]. In Europe, a waist circumference of over 88 for women and over 102 cm for men is a sign of abdominal obesity [7] .

1.1.2 Metabolic syndrome

"Metabolic syndrome (MS) is considered a medical condition caused by over-nourishment and reduced physical activity as a consequence of industrial revolution and affluence, being the most frequently referred disorder in the recent literature. However, there is no general agreement regarding its denomination, and various synonyms exist in the literature such as Wohlstands syndrome, plurimetabolic syndrome, hormonal metabolic syndrome, syndrome X, insulin resistance syndrome, and hyperinsulinemia/insulin resistance syndrome" [8] Alberti et al. described the metabolic syndrome as a complex of interrelated risk factors for morbidity and mortality of cardiovascular disease (CVD) and diabetes [9]. Even though there is no common definition for metabolic syndrome, there is a consensus in the medical field that the term metabolic syndrome is acceptable for the condition of the presence of multiple metabolic

risk factors for CVD and diabesity [7]. All major health organizations deal with dyslipidemia with an increased plasma TAG (Triacylglyceride) concentration, decreased HDL (High density lipoprotein) concentration, hypertension, impaired glucose tolerance, and overweight (Table 1).

Metabolic risk factor	World Health Organization	NCEP ATP III (2001)	AHA/ATP III(2004)	International Diabetes Federation 2005
Insulin Resistance	Required (Impaired fasting glucose, impaired glucose tolerance, T2D)+two other risk factors	Not required; any three of the five risk factors	Not required; any three of the five risk factors	Not required
Obesity	Waist-to-hip ratio ≥0.9	WC ≥ 102 cm	WC ≥102 cm	Required + two other risk factors, waist circumference 94 cm
TAG	≥150 mg/dl	≥150 mg/dl	≥150 mg/dl	≥150 mg/dl
HDL	<35 mg/dl	<40 mg/dl	<40 mg/dl	<40 mg/dl
RR	>140/90 mm Hg	>130/90 mmHg or on medication	>130/90 mmHg or on medication	≥130/85 mmHg
Glucose	IGF, IGT, or T2D	≥110 mg/dl	≥100 mg/dl	≥100 mg/dl
Other	Albumin-to-creatinine ratio >30 mg/g	Includes T2D	Includes T2D	Ethnic cutoffs for WC

Table 1: Different definitions of metabolic syndrome by the different organizations (3).

The metabolic syndrome is not an absolute risk indicator or a score like PROCAM (Prospective cardiovascular Münster) that could be applied in order to calculate the risk, because it does not contain many of the factors that determine absolute risk, for example age, sex or cigarette smoking [7]. “In the absence of CVD or diabetes, the metabolic syndrome is a predictor of these conditions. Once CVD or diabetes develops, the metabolic syndrome is often present, and the number of components of the metabolic syndrome contributes to disease progression and risk” [7]. Recently the WHO proposed the waist-to-hip (W/H) ratio [10] and the NCEP (National Cholesterol Education Program) proposed waist circumference as overweight criteria [10], but the International Diabetes Federation (IDF) suggests that obesity can be assumed at a BMI over 30 without measuring waist circumference. The different organizations have now agreed on waist circumference as a common standard [7],

[11]. In 2005, the IDF and the American Heart Association/National Heart, Lung, and Blood Institute (AHA/NHLBI) attempted to reach an agreement for the different clinical definitions of the metabolic syndrome. However, despite all efforts, a common agreement is far beyond reach. The IDF dropped the WHO requirement for insulin resistance but instead declared abdominal obesity to be one of the five factors required in the diagnosis [12]. Abdominal obesity can be determined by focusing on waist measurement as a simple screening tool. The remainder of the criteria was identical to those provided by ATP III. The AHA/NHLBI did not list abdominal obesity as a required risk factor, but slightly modified the ATP III criteria.

Recently, IDF and AHA/NHLBI representatives held discussions to resolve the remaining differences between their definitions of the metabolic syndrome [7]. Both sides agreed that abdominal obesity should not be a prerequisite for diagnosis, but included as one of five criteria, so that the presence of any three out of five risk factors constitutes a diagnosis of metabolic syndrome, as ATP III criteria have suggested before [7]. The WHO defines two levels of abdominal obesity in Europe depending on the risk for metabolic complications. There is an increased risk for waist circumferences of ≥94 cm in men, but the risk is substantially higher at ≥102 cm [7]. In this work, the IDF criteria for group stratification were applied. Our patients were exclusively from Europe. The IDF proposes reference values for the waist circumference adjusted to ethnicity. If waist circumference is used as mandatory criterion, makes no difference for our distribution into the groups.

1.2 Lipid metabolism

1.2.1 Normal lipid metabolism

Lipophilic lipids need transport vehicles. Those transporting lipoproteins are particles classified by their density. They contain neutral lipids (Cholesterylesters, Triacylglycerides) in their core and polar lipids (phospholipids, fatty acids, sterols) at the surface. Triacylglyceride (TAG)-rich lipoproteins originate either from the intestine (chylomicrons) or from the liver (VLDL) [13]. Their specific apolipoproteins permit their characterization. ApoB-48 is a specific apolipoprotein for chylomicrons and their remnants [14]. Chylomicrons (CM) transport exogenous TAGs. The lipoproteins undergo lipolysis in the circulation, thereby delivering fatty acids to tissues [13]. After reaching the plasma compartment, the chylomicrons release their apo A-I content during lipolysis and swap it for apo-CII [15] (Figure 3).

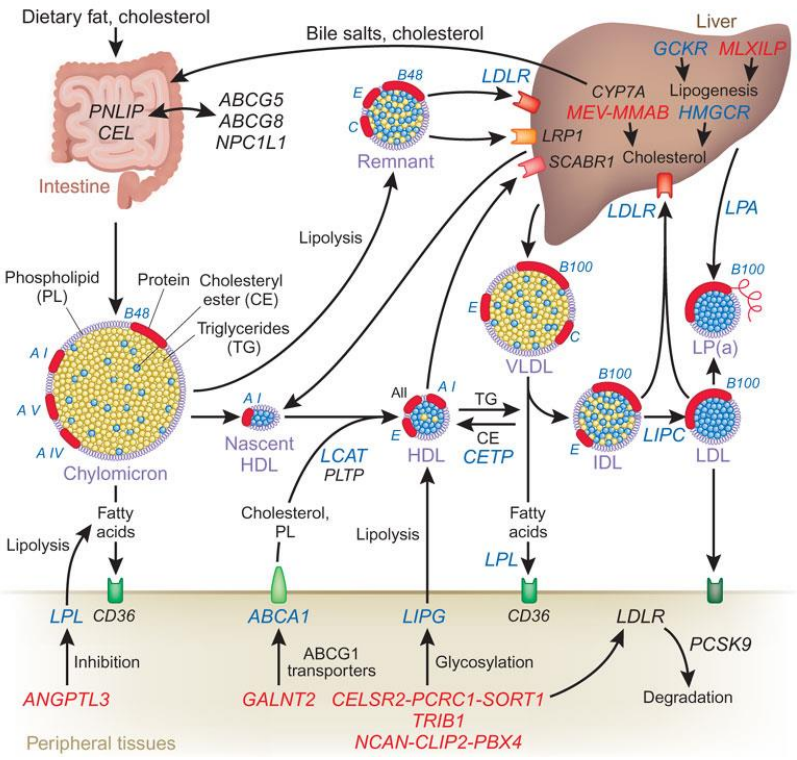


Figure 3 [13] **Lipid transport:** The TAG-rich lipoproteins are chylomicrons and VLDL. Chylomicrons are secreted by the intestine and carry the chylomicron-specific apolipoprotein apoB-48. They are split by LPL into small remnants which can be absorbed by the liver. VLDL are secreted by the liver which secrets the apolipoprotein apoB-100. LDL, a cholesterol-rich protein, is formed out of VLDL and by the liver. Since its half-life is longer than that of VLDL, the apoB-100 in plasma is mainly in LDL. HDL's main apolipoprotein is apo A-I. Apo A-I is secreted by the liver and intestine. HDL is secreted by the liver and can carry more than one apoA-1, unlike LDL which only carries one apoB-100. HDL picks up cholesterol from the cell and transports it in the form of ester. It is able to make an exchange with VLDL by taking up TAGs from VLDL. Since TAG-richer HDL has a shorter half-life than the other HDLs, the HDL concentration in plasma negatively correlates with the TAGs concentration.

This enables them to bind to endothelia-associated LPL (lipoprotein lipase) and to degrade their TAGs [16]. Chylomicrons have a half-life of approximately 5 minutes [16], whereas CM remnants have a much longer half-life time [16]. Chylomicron remnants and about half of the VLDL remnants are alternatively taken up by the liver [13]. Excess fatty acids and diglycerides in the liver induce very low density protein (VLDL) synthesis and secretion [16]. VLDL and CM are both degraded and generate the HDL precursor [16]. HDL is formed in the circulation from lipid-poor and protein-rich lipoprotein particles secreted by the liver and intestine and formed from surface components shed during the lipolysis of TAG-rich lipoproteins [13]. VLDL remnants are also metabolized to IDL and cholesterol-rich LDL, which constitutes the main cholesterol-carrying lipoprotein in humans [13]. For this cycle, LPL activity is essential and VLDL and chylomicrons compete for LPL-dependent acyl glycerol degradation [16]. Over 65% of the LDL is degraded after binding to LDL receptors in peripheral cells and hepatocytes. 15% are degraded by scavenger pathways in blood vessels.

Macrophages, which are also called scavenger cells because they carry scavenger receptors, take up chemically modified LDL such as oxidized LDL [15]. Increased levels of oxidized LDL are highly atherogenic [17]. Remnants formed during the cleavage of TAG-rich lipoproteins are also atherogenic [18]. However, remnants are rapidly and efficiently cleared from the circulation by hepatocytes which catabolize them [19].

1.2.2 Disorders of lipid metabolism in diabesity

Elevated LDL can be due to a primary hereditary dyslipoproteinemia such as LDL receptor defect or impaired LPL activity, but also to a high alimentary input of saturated fatty acids (FA) [20] and cholesterol. The most common dyslipidemias are familial hypertriglyceridemia and familial combined hyperlipidemia (FCH). FCH includes intra-individual and intrafamilial variability of the lipid phenotype [21]. What characterizes FCH are elevated LDL (low-density lipoprotein) cholesterol, especially the small dense LDL particles [21], and elevated triglyceride levels [22], often combined with a high prevalence of small very-low-density lipoproteins (VLDLs). The latter are mainly associated with an increased plasma level of apolipoprotein B-100 [21]. Patients with FCH show a relatively greater increase in apoB-100 compared to the TAG increase [23]. Some patients may show a decrease in high-density lipoprotein (HDL) cholesterol plasma level [21]; [24]. Non-HDL cholesterol is elevated [25].

Stratification according to Fredrickson:

Type	Elevated lipid fraction
I	Chylomicrons
IIA	LDL
IIB	LDL and VLDL
III	ß-VLDL (E2/E2)
IV	LDL
V	VLDL and Chylomicrons

Hypertriglyceridemia can also be caused by high caloric input and increased fat and glucose intake [20]. Disturbances of the lipid metabolism can also be due to secondary diseases such as hypothyroidism, nephrotic syndrome, and cholestasis [20], which can be responsible for high cholesterol levels. Diabetes mellitus, obesity, alcoholism and terminal kidney insufficiency, on the other hand, can result in hypertriglyceridemia and low HDL levels. What is important is the distribution of LPL among tissues [26]. As Wang et al. described in 2009, a

loss of LPL in muscle cells can lead to obesity and insulin resistance in cells other than the skeletal muscle [26].

1.2.3 Adipose tissue metabolism

Adipose tissue can be divided structurally, functionally and topologically into white and brown as well as into visceral and subcutaneous fat. It is not only a storage organ, but has many different modulatory functions in the endocrine and immune system. Brown adipose tissue, which is nearly absent in adults, is essential for children in order to maintain their body temperature. It uncouples oxygen via uncoupling protein (UCP) from ATP (adenosine triphosphate) production to produce heat and ROS (Reactive Oxygen Species) [27]; [28]. In healthy subjects with normal caloric intake, the caloric output can be induced via adrenergic receptors. The increased cAMP (cyclic adenosine monophosphate) can then trigger the caloric output via activation of thyroid hormone and UCP [27]; [28]. In obese subjects, the UCP pathway is less frequently provoked due to an increase in SHP (small heterodimer partner), which itself triggers diet-induced obesity and an impaired glucose tolerance [27], [28].The white adipose tissue, which is subject of this work, contains adipocytes, preadipocytes, fibroblasts, leukocytes, macrophages and endothelial cells; it provides energy and fatty acids from its stored lipids.

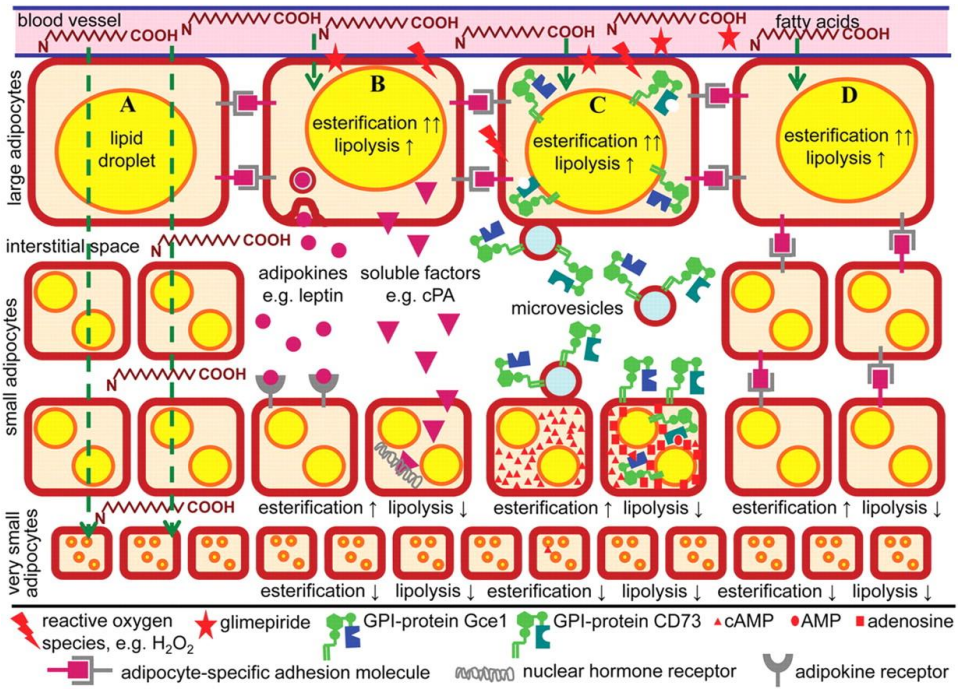


Figure 4: Communication between large and small adipocytes, involving cell-to-cell contact and signal peptides like adipokines (4). The resulting parallel upregulation of esterification (↑) and downregulation of lipolysis (↓) leads to an enlargement of the lipid droplets and the size of the adipocytes [29].

Moreover, white adipose tissue produces hormones and adipokines as well as influences different bodily functions. Large adipocytes secrete adipokines and microvesicles, which are taken up by small adipocytes, switch off lipid release and switch on storage. This process leads to a conversion into large adipocytes [29] (Figure 4).

1.3 Etiology and pathogenesis of obesity and the metabolic syndrome

The development of obesity is associated with multiple factors. The reasons for obesity can be summarized as a drift of energy homeostasis to the positive side (orexia). A positive energy balance is caused by excessive food intake, especially saturated FAs, and a lack of physical activity, which are the main reasons for obesity [30]. This shift from a balanced energy to a positive energy balance can also be the consequence of a lack of the hormone leptin due to a genetic mutation or a defect of the receptor. There can be also a defect in the α-MSH (melanocyte stimulating hormone) liberation, for example due to an enzyme defect or a lack of the receptor of α–MSH [31]. It should be mentioned that studies have found a more significant relation between MSH and type 2 diabetes than between MSH and obesity [31]. Genetic influence is responsible for 40% of the cases of obesity [32]. The energy output is influenced by basic metabolism, thermogenesis and activity. During the development of obesity, the energy regime is kept positive by the input of rich and too much food. Later on, energy input and consumption are balanced, but due to the increased mass of fat, the basal metabolic rate is elevated. Urbanization and the modern lifestyle, in which less physical activity is combined with an elevated fat content in the diet, are almost certain to lead to progressing obesity [2]. Psychosocial factors can also influence the development of obesity: anxious and depressive individuals are more likely to become obese [33]. The suggestion that a lack of sleep in youth leads to obesity has not been confirmed yet [34]. Of course, environmental factors such as the workplace may influence a person's nutrition. People who work with food, for example butchers or bakers, are more frequently affected by obesity. Notable relevance should be given to the impact of cultural and social values. Whereas in some cultures, obesity is a status symbol which makes overweight desirable, in other cultures it is not socially accepted [2], [35]. Endocrine reasons for overweight include Cushing's syndrome, Hypothyroidism or Insulinoma [36]. Changes in the mass of adipose tissue, the hypertrophy and hyperplasia of adipocytes, connected with angiogenesis and proliferation and expansion of the extracellular matrix, lead to changes of metabolic pathways [37] and epigenetic modulation [38].

1.4 Consequences and clinic of obesity and the metabolic syndrome

The first symptoms of obesity are dyspnea, pain of angles and back, fatigue, cardiac problems, edematous legs in the evening, obstipation and mental stress. The associated risks of further diseases depend on the distribution of the fatty tissue (Figure 5). Even though women have more body fat than men, their pear-shaped body fat distribution is associated with lower cardiometabolic risk in contrast to the metabolic consequences of central obesity typical in men [39]. The pear-shaped phenotype reflects the subcutaneous white adipose tissue depots just under the skin, which are mainly in the gluteal and femoral areas, but also in the waist [39]. The intra-abdominal depots including visceral adipose tissue often consist of more, but less differentiated, adipocytes [39]. "Obese patients with the metabolic syndrome generally have a visceral (apple-shaped) fat distribution and are at an increased risk of macrovascular disease, while those with peripheral (pear-shaped) obesity do not tend to have metabolic abnormalities and are at lower risk" [40]. "This difference appears to be related to the differing metabolic functions (and secretory products) of visceral adipose tissue and subcutaneous adipose tissue, as well as the fact that visceral fatty tissue drains directly into the liver" [40].

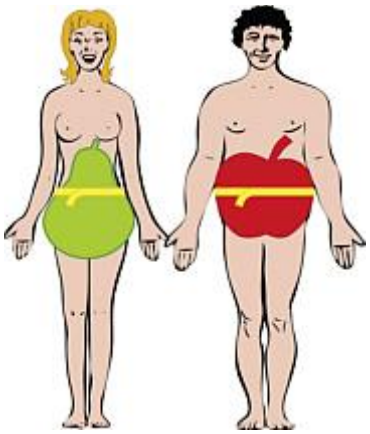


Figure 5: Different types of fat distribution with different risk for associated diseases (5).

Both phenotypes (pear and apple) differ in their predominant type of growth [39]. With age, the capability of increasing the number of fat cells does not vanish, as has been suggested, but the fat cells mostly gain in size [41]. The consequence of excess lipid accumulation, even though it is limited to 0.8 µg per cell, is devastating. The change in number, unlike the change in size, is not reversible [42]. The change of the adipocytes' size causes changes in the

composition of the cells, increases lipolysis and thus an elevation of the fatty acid concentration in the plasma, which then alters insulin sensitivity. The changes in number and size of adipocytes lead to changes in hormone and transmitter secretion and finally to a chronic state of inflammation in the entire body. Thus, the alteration of the tightly controlled compositions of the cell content causes the loss of specific functions. As a consequence, the risk of developing type 2 diabetes, hypertension, coronary heart disease, hypercoagulopathy, stroke, gallbladder disease, sleep apnea, osteoarthritis, osteoporosis and cancer is increased [41].

1.4.1 Diabetes in metabolic syndrome

Insulin synthesis

Proinsulin, the precursor of insulin, is synthesized in the rough ER of ß-cells in the pancreatic islands and plays a key role in insulin biosynthesis [43]. Proinsulin consists of the three polypeptide chains A, B and C, and their folding is ensured by three disulfide bridges [43,44]. The precursor of proinsulin is the mRNA pre-proinsulin [45] The pre-proinsulin is translated into a single polypeptide chain of polyribosomes associated with the rough ER [45]. During the transition through the rough ER, proinsulin is cotranslationally processed by the elimination of its signal sequence. Proinsulin forms its tertiary structure by intra-molecular disulfide bonding. The precursor was discovered as early as in 1967, and it changed the perception of hormone bio-synthesis [43]. Proinsulin is separated from its C-peptide, which is secreted in equimolar concentration as insulin, by the endocrine part of the pancreas [45]. The plasma concentration of C-peptide is used as a biomarker for endogenous insulin secretion.

Insulin secretion and impaired glucose tolerance

An increased plasma concentration of glucose, certain amino acids and acetoacetate, which is a product of ß-oxidation and fatty acids, up-regulates insulin secretion [46]. In the liver and pancreas, glucose is taken up via GLUT2 (glucose transporter) depending on the glucose concentration. But GLUT4, which is responsible for glucose uptake in adipose and muscular tissue, has also been identified in pancreatic cells [47]. GLUT2 only has a low glucose affinity (Km 17mM) and the velocity at which glucose is transported in the cell by GLUT2 cannot be increased by the blood glucose concentration, but the velocity by which the glucokinase works depends on glucose concentration and can be increased between blood glucose levels of 3 and 15 mmol/l [48]. The exocytosis of insulin containing granules of ß-cells from the

pancreas is regulated via mitochondrial ATP/ADP ratio, which increases in the presence of glucose [48]. Elevated ATP levels provoke the closing of potassium channels [48].

A closure of potassium channels results in a depolarization of the cellular membrane, which causes the calcium channel to open and finally an exocytosis of insulin granules [48]. The secreted insulin induces GLUT4 incorporation in the membranes of fat and muscle cells, where glucose is rapidly taken up via passive diffusion. This type of transport is called insulin-dependent glucose uptake [48]. This first phase of the insulin response lasts 2-5 minutes [49]. Insulin increases the V(max) of glucose elimination in the blood [48]. The danger of an insulin overdose lies in the increased insulin-dependent glucose absorption by muscle and fat tissue, whereas insulin-independent cells in the CNS may develop hypoglycemia. In the muscle tissue, glucose uptake results in increased glycolysis and glycogen synthesis; in the fat tissue, the elevated availability of acetyl-coA, which is dependent on the activity of the pyruvate dehydrogenase complex, results in a de novo synthesis of free fatty acids and their incorporation in TAG and thus increased lipid storage [48]. Insulin is an anabolic hormone which reduces lipolysis and activates liponeogenesis in the liver and in fatty tissue. The reduction of lipolysis implies lower free fatty acid (FFA) concentration levels in the plasma. In diabetics, the elevation of FFA in the plasma has lipotoxic effects [49]. Glucose tolerance can be maintained for a long time in obese individuals despite a decreased sensitivity to insulin. This is due to an increase of insulin secretion. The normal insulin secretion works by calcium-dependent exocytosis after an ATP-triggered cell depolarization. Insulin secretion can be either directly influenced by glucose or in a second step, which means by the metabolic intermediary products when pyruvate is converted to triglycerides. This mechanism is defined as “glucose-stimulated insulin secretion” (GSIS). Glucose-stimulated insulin secretion also has a long-term impact on glucose transport and utilization and is itself positively controlled by fatty acids and lipids in the plasma. For this second step of insulin secretion, the amplification phase is mediated by citrate, which is exported from mitochondria [50]. Citrate is a substrate and the resulting products are short-chain FAs which induce insulin secretion [50]. Thus, glucose increases its own insulin-dependent uptake with an autocrine amplifying mechanism, glucose-stimulated insulin secretion. Especially under fasting conditions, endogenous fatty acids are important for the secretion of insulin. The maintained insulin secretion is important in order to oppose lipolysis and its consequences, lipotoxicity and ketoacidosis. Exogenous, especially long and saturated, FAs can also amplify insulin secretion by GSIS [50]. This is necessary in order to develop an acute hyperinsulinemia to oppose the reduced glucose uptake. In the insulin-

resistant state, GSIS is not enhanced but even reduced by the chronically elevated fatty acid level. The exaggerated lipolysis leads to lipotoxicity and a false distribution of triglycerides as well as an insulin resistance of the affected organs. PPARs decrease the concentration of lipids in the blood via RXR, thereby opposing lipid-dependent insulin resistance. Thus they lower the need of increased insulin secretion. A chronic elevation of FA is a problem. In this state, GSIS is reduced, but the insulin secretion by other mechanisms is not affected or even enhanced.

From impaired glucose tolerance to type 2 diabetes

In impaired glucose tolerance, the first phase of insulin secretion is reduced, which results in high postprandial glucose levels, whereas fasting glucose levels are normal. Later, glucose levels are also elevated under fasting conditions, but basal insulin secretion can be kept normal by compensation. In manifest diabetes, basal insulin secretion can also be reduced. Elevated plasma fatty acids as well as high insulin levels in the blood increase insulin resistance. High insulin levels in the blood are responsible for less insulin production, thus blood glucose remains high. The insufficient supply of the tissues with glucose leads to an increased glucose production by the liver. Since the hunger cannot be satisfied, the glucose intake is elevated as well. Insulin resistance is one of the main reasons for a higher glucose intake and thus obesity. It is triggered by inflammation, stress and free fatty acids.

1.4.2 Inflammation in the metabolic syndrome

Inflammation can be modulated via adipokines (signaling proteins that derive from the adipose tissue); therefore an increased volume of fatty tissue is associated with a chronic state of inflammation. The first link between obesity, insulin action and chronic inflammation involving TNFα was established in 1993 by Hotamisligil et al [51]. The workgroup named the chronic low-grade inflammation associated with overnutrition ”meta- inflammation” [52]. This meta-inflammation lowers adiponectin and insulin sensitivity, and it prevents the differentiation of preadipocytes into adipocytes. On the other hand, meta-inflammation is accompanied by a chronically increased macrophage concentration [52] and ER stress. The complex mechanisms have not been fully understood so far, but e.g. IKKβ are involved in the inflammation pathways, which results in the activation of NF-κB [53] as a mediator of TNF-induced insulin resistance as well as in the activation of serine/threonine protein kinases.

1.5 Adipokines

Since adipokines are cell-to-cell signaling proteins that derive from the adipose tissue, altered blood concentrations and dynamics reflect an altered physiology in subjects with changes in adipose cell number, volume and composition. Their modulating role in obesity, insulin resistance and inflammation have already been reported previously [54]. Their origin and site of action are graphically displayed below (Figure 6, Figure 7 Figure 9).

1.5.1 Leptin

Leptin is mainly expressed by adipocytes. It is also produced in the placenta, bone marrow, stomach, muscle and brain. Its main function is controlling the appetite. Leptin opposes adiponectin and acts as a proangiogenic and proinflammatory agent [55]. It stimulates the proliferation of circulating monocytes in vitro and up-regulates the expression of activation markers on monocytes and neutrophils. Leptin stimulates ROS production [55]. Moreover, Leptin regulates lipid metabolism (Figure 6). Leptin levels are increased in obese subjects. Subjects with metabolic syndrome often prove insufficient regulation due to leptin resistance.

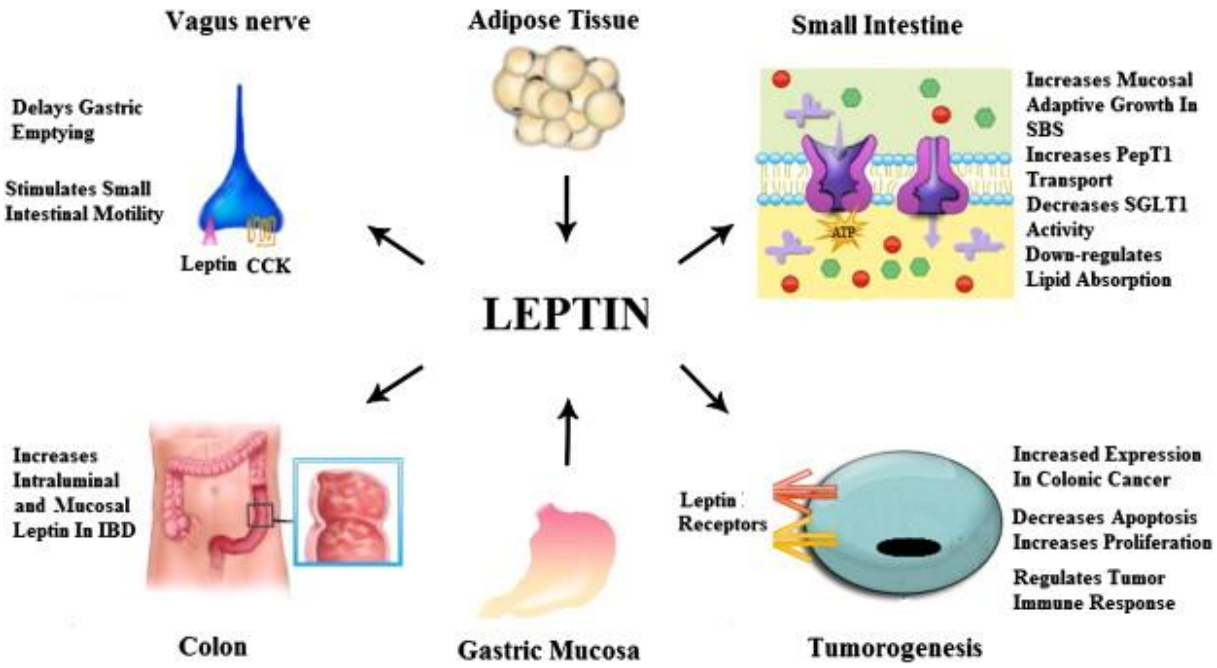


Figure 6: The **peripheral action of leptin**. Leptin is mainly secreted by adipose tissue and the gastric mucosa. It regulates appetite, inflammation and lipid absorption (Image taken from [56]).

1.5.2 Adiponectin

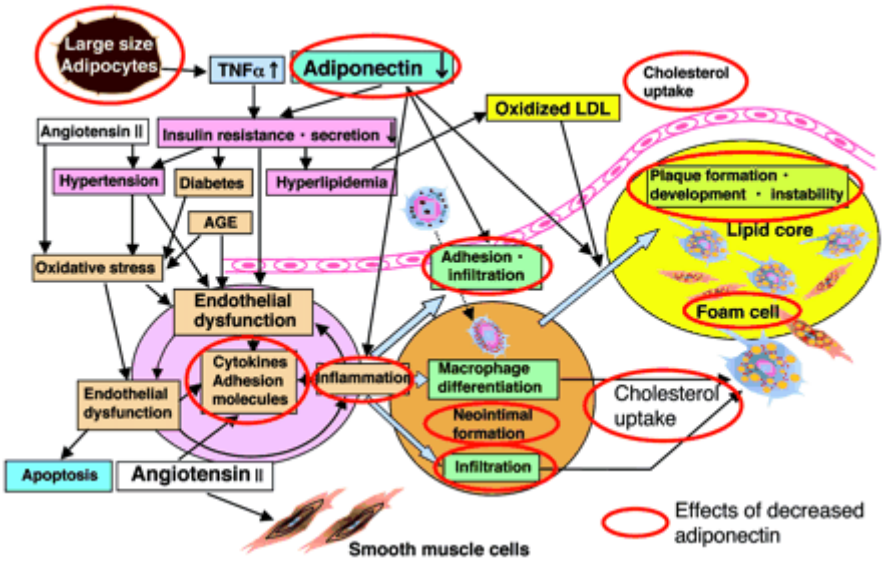


Figure 7: Effects of a decreased adiponectin level in the blood and the association with plaque formation (6).

Because of its exclusive secretion by adipocytes and its pleiotropic positive functions in reducing the diseases associated with obesity, it is a suitable biomarker for the risk of metabolic syndrome. Scherer et al. showed that, in mice, adiponectin is capable of boosting the sensitization of the liver to circulating insulin. The decrease of adiponectin levels in obese subjects leads to inflammation and insulin resistance and thus to hyperlipidemia. Inflammation directly leads to the formation of foam cells and, due to hyperlipidemia, increased levels of oxidized LDL support foam cell formation [57]. Increased oxidative stress leads to endothelial dysfunction. The result is plaque formation and an increased risk for CAD (cardiac artery disease) (Figure 7).

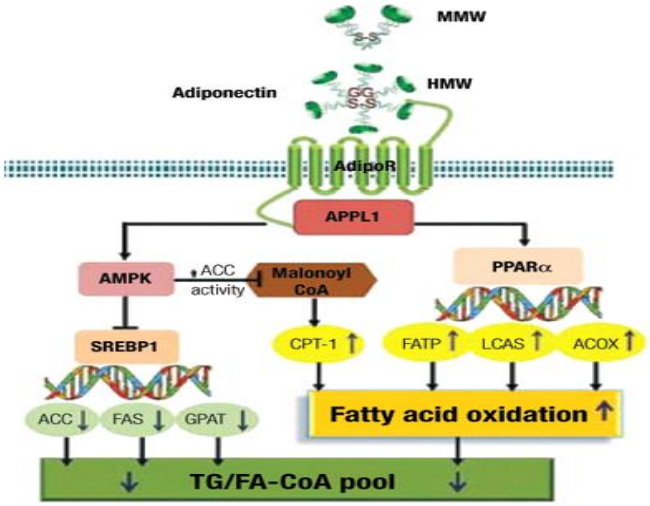


Figure 8: Adiponectin increases fatty acid oxidation [58].

Figure 8 depicts the role of adiponectin in hyperlipidemia. It describes the effects of adiponectin on signaling pathways by mediating ß-oxidation and decreasing triglyceride concentration.

1.5.3 Omentin

Omentin is especially expressed by visceral adipose tissue [59]. The plasma concentration of omentin negatively correlates with glucose tolerance [59] and positively correlates with endothelium-dependent vasodilation (Figure 9). Plasma omentin levels negatively correlate with carotid intima-media thickness, which is a marker of early atherosclerosis [59]. "Low levels of circulating omentin are also associated with the prevalence of coronary artery disease These data suggest that omentin may represent a biomarker for not only metabolic disorders, but also cardiovascular diseases" [59].

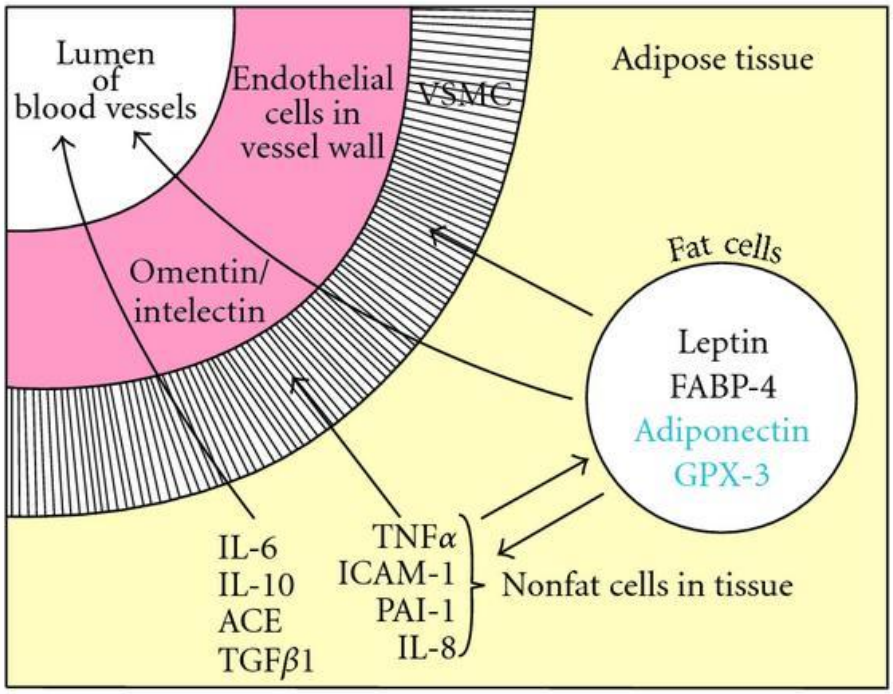


Figure 9: Omentin, leptin and adiponectin have modulating effects on the vessel wall (VSMC = vascular smooth muscle cells). Omentin/intelectin is shown as being secreted by the endothelial cells of the blood vessels of omental but not subcutaneous fat (7).

1.6 Lipids

Lipids are classified into classes such as triglycerides (triacylglycerols), fatty acids, phospholipids, plasmalogens, sphingolipids, and sterols. Each lipid class contains numerous

lipid species that differ in various aspects e.g. defined by fatty acid chain length and desaturation. In any eukaryotic cell, more than 1000 different lipid species exist [60]. When challenges in the environment destroy homeostasis, the maintenance of the physiological functions is usually restored. But a change of 10% of lipid content can already have significant consequences [61]. Due to the concentration of stressors, the response to the environment can be inefficient. This phenomenon is called “allostatic load” [61]. An example for an allostatic load would be an ablation of SREBP1 (sterol regulating binding protein), which preferentially regulates the FA metabolism. In this case, SREBP2 would be automatically up-regulated, which might lead to an inappropriate cholesterol accumulation [61]. Another example is elevated insulin secretion in persons with insulin resistance. The allostatic regulations might compensate certain failures, but the symptoms of damage and allostatic overload can progressively lead to symptoms. Oresic noted that these allostatic responses could be used as early biomarkers to quantify the risk for certain diseases [61].

1.6.1 Plasmalogens

Plasmalogens constitute 18% of the total phospholipid mass in humans [62]. They are synthesized in the liver and transported in the blood stream bound to proteins. The majority of PE-plasmalogens (ethanolamine plasmalogens) in the plasma are transported in HDL particles [62]. The ethanolamine plasmalogens are important in this study because of their antioxidant effects [63]. The quantity of plasmalogens in a cell reflects the quantity of peroxisomes in that cell, because peroxisomes are the location of plasmalogen synthesis. Ethanolamine plasmalogens have a half-life of 3 hours [62]. And contrary to other lipids, they revealed a high level of variation between different tested individuals, which could make them an interesting marker for peroxisome function [62]. It is known that with age, plasmalogen synthesis in humans decreases [62]. A lack of plasmalogens is associated with ER stress, the “unfolded protein response”, which, as a last consequence, leads to apoptosis. Plasmalogens also serve as a reservoir for PUFAs and a reduced quantity of plasmalogens could be associated with a reduced quantity of ω-3 fatty acids species, which serve as anti-inflammatory messengers, and of adipogenesis-regulating fatty acids [62]. Since a decreased number of plasmalogens is associated with diseases such as diabetes and obesity, our laboratory proposed to use plasmalogen species as early stratifying biomarkers and indication for disease and therapy [62].

1.6.2 Fatty acids

The fatty acid composition in serum is an important determinant of the metabolic syndrome. Kotronen et al. showed that there is a close connection between the quantity of saturated fatty acid in triglycerides and diabetes. His work group has already proposed some species as biomarkers [63]. They found out that serum TAG molecules containing saturated and monounsaturated fatty acids, such as TAG (16:0/16:0/18:1) and (16:0/18:1/18:0), correlated positively, whereas those containing mono- and di-unsaturated FAs such as (18:1, 18:2) correlated negatively with impaired glucose tolerance, hepatic lipogenesis and waist circumference. However, saturated TAGs only correlate positively with features of insulin resistance in VLDL, IDL and LDL, not in HDL. Free FAs only have a half-life of 12 minutes.

Saturated long-chain fatty acids can up-regulate ceramide production, whereas short-chain unsaturated fatty acids protect ß-cells from the toxicity of ceramides. Polyunsaturated fatty acids (PUFAs) can be classified in ω−3 fatty acids and ω−6 fatty acids, and in the westernized diet the predominant dietary PUFAs are ω−6 fatty acids [64].

1.6.3 Phosphatidylcholine and Lysophosphatidylcholine

Phosphatidylcholine also counts among the glycerophospholipids. Lysophosphatidylcholine (LPC) is a signaling molecule with functions in cellular proliferation and inflammation [65]. A negative correlation between BMI and LPC has been published [65]. The exposure of adipocytes to LPC in vitro has caused their improved glucose tolerance, thus LPC might be involved in glucose regulation. Reduced LPC levels in obese subjects might contribute to insulin resistance [65]. Enhanced LPC levels may be an early marker for development of type 1 diabetes [66].

1.6.4 LPA

Lysophosphatidic acid (LPA) is a phospholipid, which consists of a single fatty acyl chain, a glycerol backbone and a free phosphate group [67]. By binding to specific G-protein-coupled receptors, LPA activates multiple signal transduction pathways, including diverse processes such as wound healing, brain development, vascular remodeling and tumor progression [67].

1.6.5 Sphingomyelin and ceramides

Sphingolipids derive from the sphingosine backbone. They are components of cellular membranes, enriched in membrane microdomains (rafts) [68]. They can be found in nerves and are important for signal transduction. Those which are interesting for our study are the sphingolipids belonging to the main group, such as ceramide and sphingomyelin. Ceramide is the simplest sphingolipid, consisting of one free fatty acid (palmitate) and serine to form the sphingosine base. The intracellular elevation of ceramides is stimulated by stressors and inflammation. Ceramides inhibit cell growth, proliferation, differentiation and induce apoptosis. An equilibrium of ceramide and sphingosine-1-phosphate is crucial for the balance of death and survival [69]. It is known that sphingosine is elevated in the serum of type 2 diabetes subjects compared to healthy controls. Ceramide can contribute to insulin resistance by inhibiting GLUT4 translocation, glycogen synthesis, altering pancreatic cells and insulin-stimulated glucose uptake [69]. Risk of atherosclerosis is associated with high levels of sphingomyelin, which is overexpressed in obese subjects due to the higher levels of serine palmitoyl-transferase in the sera of obese subjects compared to lean ones.

1.7 Current biomarkers

The clinical utility of a biomarker depends on its ability to account for a significant proportion of the disease's evaluation, its accuracy and reliability and on its ability to provide good sensitivity and specificity and a high predictive value [70]. The well-established biomarkers for the metabolic syndrome are already incorporated into the diagnostic criteria. Additional potential biomarkers for the metabolic syndrome have been identified. The predictive values for the risk of metabolic syndrome of inflammation parameters, e.g. CRP [71], or adipokines such as ghrelin [72], adiponectin [73] and leptin [74,75] have been published recently. Elevated fasting CRP concentrations and decreased adiponectin levels as well as elevated postprandial triglycerides are not only a hallmark for the metabolic syndrome, but are also independent predictors of CVD risk [76]. In previous studies, only the basal values have been taken into account, but the dynamic has not yet been compared between a metabolic syndrome group and a control group.

1.8 Aim of the study

The aim of this study was to identify novel biomarkers for diabetes, hyperlipidemia, atherosclerosis and CVD risk which might have prognostic value. Since those diseases are

connected with the metabolic syndrome, the risk for those diseases was measured based on the risk of having metabolic syndrome. To this aim, we analyzed the differences between three groups: control (none of the criteria defining metabolic syndrome), risk (two of the risk factors) and metabolic syndrome as defined in Table 2. The aim was to test the hypothesis that metabolic syndrome subjects have an acutely and chronically altered lipid metabolism compared to the control group, suggesting an altered postprandial lipidomic response in diabesity. The examination was carried out by observing the effects of an oral fat tolerance test on those body compartments which are associated with obesity.
For the oral fat tolerance test, the subjects were given a drink consisting of 66% fat and their plasma samples were compared to the fasting plasma sample 2, 4, 6 and 8 hours after the drink. Adipokines, inflammation markers and signaling proteins as well as lipids and lipidomics of the adipose tissue, blood compartments, kidney and bone were measured on the assumption that persons with metabolic syndrome react differently to high fat load than healthy subjects, because their metabolism is acutely and chronically altered. For this investigation, the dynamic of blood components was compared between different groups (named control, risk and metabolic syndrome) according to their diet and risk factors associated with obesity. With this work, we hope to contribute to the definition of a new multimarker panel – with greater sensitivity and specificity than the current ones – in order to screen and monitor diabesity patients with the metabolic syndrome, assess their courses of disease and predict the outcome of the disease.

2 Materials and Methods

2.1 Materials

Consumables	Manufacturer
Inlet liners Carbo Frit	Retek
Silica HPTLC plate, phospholipid standards	Avanti Polar Lipids
Test tubes for diluting samples	Eppendorf

Reagents	Manufacturer
Acetylchloride	Merck
Ammonium acetate and acetyl chloride	Fluka
Cholesterol (95% purity)	Sigma
Cholesteryl ester standards (98% purity)	Cambridge Isotope Laboratories Andover
2-13C acetate	Cambridge-Isotope Lab
EDTA	EDTA
Ethanol	Merck
FA standard D3C-16:0	Sigma
FAME standard	Lordan
HCl 1N	Sigma
n-Hexane	Merck
Medical Isotopes (99%purity)	Pelha
Methanol	Merck
NaOH 10N	Sigma
PHMB	Sigma
Potassium phosphate buffer 0,1M pH 7,4	Sigma

Instrumentation	Manufacturer
Agilent 110 binary pump	Agilent
CAMAG TLC scanner	Camag
HTS PAL auto sampler	CTC
GCMS-QP 2010 detector+autosampler+PTV (programmed T vaporizer)	Shimadzu
Graduated cylinder for dilution buffer	Braun
Triple quadruple mass spectrometer with electrospray ionization source	Waters
Vortex Mixer	Sigma
Orbital microplate Shaker	Sigma

Immunoassays	Material	Manufacturer
ApoB-48	Serum	Shibayagi
FGF-23	Li-Hep-Plasma	Immutopics
Leptin	Serum	Bio Vendor
Ghrelin	Li.-Hep.	SPI bio
Omentin	Li-Hep-Plasma	Bio Vendor
Su-Par	Li-Hep-Plasma	ViroGates

2.2 Selection of subjects and stratification

A small presentation of our study was prepared with contact dates on business cards and we placed ourselves in the city once, another time at the University Hospital Regensburg and at Regensburg University. The project was explained to a random sample of people who passed and who were asked if they were interested in taking part as volunteers. Then we checked whether they met the inclusion criteria. As an incentive for participating, we offered the volunteers their laboratory parameters with interpretation and a treatment recommendation if necessary. We handed out our cards and asked them to call us for an appointment. Additionally, the medical doctor in cooperation with the department of cardiology agreed to put us in contact with patients meeting our inclusion criteria. We recruited 70 healthy male volunteers from town, University Hospital and University. 3 of them decided to abort the experiment prematurely without providing reasons. The 67 healthy volunteers were stratified in three groups according to IDF criteria (Table 2): the metabolic syndrome group contained subjects which fulfilled at least three of the criteria, the risk group consisted of subjects which fulfilled at least one but less than three criteria, and the control group contained subjects which did not show any of the risk factors from the IDF criteria. Inclusion criteria were ages between 35 and 70 and male gender. The IDF definition of the metabolic syndrome is that a subject is afflicted by the metabolic syndrome if abdominal obesity and two or more of these criteria are fulfilled (Table 2).

Parameter	Reference range
Elevated triglycerides	≥150 mg/dl or drug treatment
Reduced HDL cholesterol	<40 mg/dl for males or drug treatment
Elevated blood pressure	Systolic ≥ 130 mmHg and/or diastolic ≥ 85 mmHg or drug treatment
Elevated fasting glucose	≥ 100 mg/dl or drug treatment
Elevated waist circumference	men ≥ 94 cm

Table 2: **IDF criteria for metabolic syndrome** [77] To fit into our metabolic syndrome group, the subject had to fulfill three (obesity+2 of the criteria) or more of the five criteria.

Subjects were excluded if they had a history of malignant disease or disorders in their gastrointestinal tract.

2.3 Design

The approval of the hospital ethics committee (no. 08/119) was obtained. Each patient who participated in our study especially had to be informed of the potential side effects of the drink (nausea immediately or diarrhea 1-2 hours after drinking), sign an informed consent (Annex 1) and complete a questionnaire (7.2) with questions on medical history, physical activity, eating habits, medication, allergies and family history. The oral fat tolerance test is similar to the oral glucose tolerance test (OGTT).

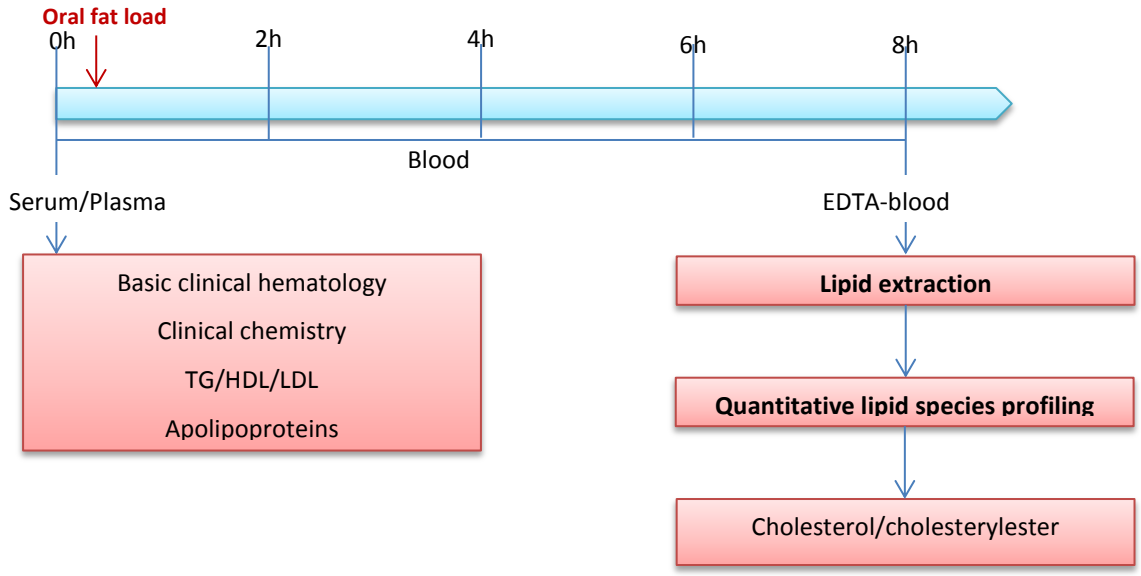


Figure 10: Procedure. Blood samples were taken following strict 12-hour fasting at time points 0, 2, 4, 6 and 8 hours after drinking the high caloric shake. The samples were analyzed for their lipidomic profile, as well as for cellular parameters and serum cytokines.

The oral fat tolerance test (OFTT) was carried out as suggested by Hanefeld group (Dresden) with a minor modification of the variant by Henkel E. et al. (2005) [78]. As described in Figure 10 blood samples were taken from the antebrachial vein, following a strict 12-hour fasting period at time points 0, 2, 4, 6 and 8 hours after drinking a high caloric shake. The shake consisted of 250 ml cream with 100 ml fruit juice in combination with 60g sugar. Sugar was added to adjust the combined fat-glucose content. The fruit juice was added to the high-fat cream to neutralize the metallic taste of the pure drink and to make the drink appetizing. In total, the drink contained 3% protein, 66% fat and 32% carbohydrate, adding up to a total energy of 1016 kcal (Figure 11).

	Kcal	Protein [g]	Fat [g]	Carbohydrate [g]	PUFA [g]	SUFA [g]	Total FA [g]
250ml/286g milk cream	720	6.2	75	8	2.8	22.6	45.5
100ml/100g fruit juice	53	0.3	0	12	0.2	0	0.1
60g white sugar	243	0	0	60	0	0	0
Total content	1016	6.5 (4.02%) 3% kcal	75 (46%) 66% kcal	80 (49.5%) 32% kcal	3	22.6	45.6

Figure 11: Composition of the oral fat drink according to manufacturers' declaration.

We first measured the patients' blood pressure, waist and hip circumference. Waist and hip circumferences were measured in a standing position between the lower costal margin and the iliac crest in the narrowest section. Hip circumferences were measured at the level of the greater trochanter. Blood pressure was measured five minutes after relaxing in an either sitting or lying position. Immediately before the drink was handed out, we inserted an antebrachial cannula for blood sampling or, where this was not possible; we drew blood with a butterfly needle. After the first withdrawal of fasting blood, the blood was immediately transported to the laboratory, and the drink then had to be consumed within a maximum of ten minutes. During the experiment, the participants were advised to stay in the hospital building and were only allowed to drink water. We took a sample of every drink; ten samples were randomized to control the fatty acid composition of the drink. Six tubes were filled with every blood collection: 4x EDTA, 1x lithium heparin, 1x serum. The samples were analyzed for their lipidomic profile, as well as for cellular parameters and serum cytokines. Special emphasis was put onto adipokines which might be regulated differently in persons with metabolic syndrome compared to the control group. Samples from each drink were aliquoted and stored at -80°C for further analysis.

2.4 Laboratory parameters

2.4.1 Lipid mass spectrometry

Mass spectrometry was developed by J.J. Thomson in 1910 [79]. It is an analytical laboratory technique to separate the components of a sample according to their mass [79]. Since the 1950s, gas chromatography has been coupled with mass spectrometry [80]. In this case the samples are vaporized into gas and then ionized. The quantity of ions at different deflections

is plotted as a spectrum of different masses [79]. Figure 12 shows our modern instrument for quadruple mass spectrometry with the CTC PAL HTS autosampler.

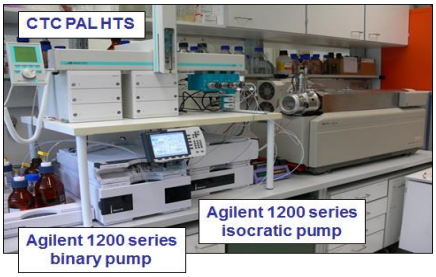


Figure 12: The laboratory instrument, an instrument for mass spectrometry with autosampler and Agilent pumps, API 4000 QTRAP. Samples were vaporized and ionized and plotted as a spectrum of different masses. Source: Gerhard Liebisch, University of Regensburg.

.

Figure 13 shows a short overview of the workflow of analyzing the different lipid classes.

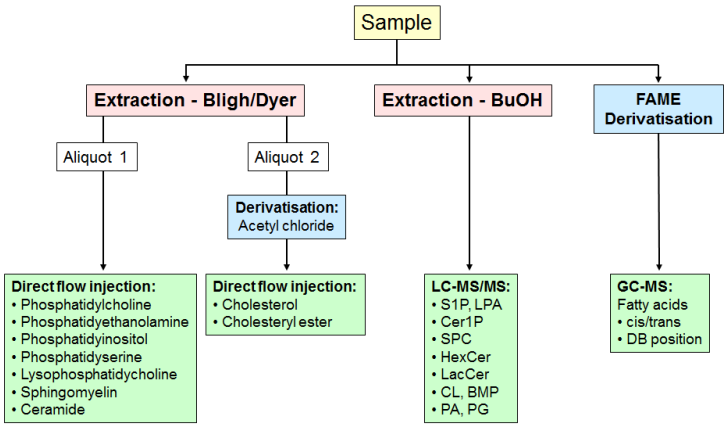


Figure 13: Strategies for lipid analysis. On the left, the separation and identification of the phospholipids that were prepared by the method of Bligh and Dyer and then separated by electroflow separation is depicted, on the right the identification and quantification of the fatty acids is shown. Here, the first step is preparation, the second GC-MS. Source: Gerhard Liebisch, University of Regensburg.

Parameter	Preparation procedure	Reference value
Ceramides	Bligh and Dyer	
Fatty acids	FAME	0.3-1 mmol/l
LPA		0.1 µM
LPC		< 150 µM
Phosphatidylcholine	Electrospray ionization tandem mass spectrometry with isotope correction algorithm	
Plasmalogens	Bligh and Dyer	
SPM	Electrospray ionization tandem mass spectrometry with isotope correction algorithm	

Table 3: Parameter list of mass spectrometrically tested substances and reference values. Values found in [81], [82], [83].

Fatty acid analysis

Fatty acids (total hydrolysis and non-esterified fatty acids [NEFA]) were measured as methyl esters by GC/MS after derivatization according to methods published by Ecker, Liebisch et al. [84]. Fatty acid methyl ester (FAMEs) were prepared by transesterification in one step reaction with acetyl chloride and methanol according to the protocol of Lepage and Roy [85]. Cyanopropyl polysilphenyl-siloxane columns are preferred to wax columns as they have a high temperature stability [85]. The inert gas helium was used as mobile phase and carrier. The FAMEs were transported by the helium, which interacted with the solid phase. Each compound had its own retention time. The comparison of retention times with the standard analytics made it possible to identify the peaks. And with mass spectrometry as a detector, the contents of the samples could be determined. Sensitivity is gained by using a programmed temperature vaporizer, which can even be used for samples with low concentrations [85]. **Preparation:** Fatty acid methyl ester (FAMEs): 10µl plasma were methylated in PTFE screw capped pyrex tubes [85]. 1µl of C13:0 for quality control and 1µl C21:0 iso as internal standard were added in 50 µl Methanol [85]. Derivatization was performed by shaking 2ml of methanolic acetylchloride (10%) and 50µl n-hexane in a 95°C water bath for 1h [85]. At room temperature, 5ml of a 6-percent potassium carbonate solution were added. 100µl of the top-layer were transferred into a 500µl autosampler vial [85]. **Gas chromatography coupled to mass spectrometry:** An aliquot of 1µl FAMEs was separated by BPX70 column coated with 70% cyanopropyl polysilphenyl-siloxane using a GC 2010 coupled to a GSMS-QP2010 detector. PTV run in split mode 1:20 for 3s switched for 1.3min to the splitless mode and a split ratio of 1:100 until the end of the run [85]. The liner was packed with CarboFrit^{TM} [85]. The temperature was 50°C for 0.75min, then the temperature was increased by 40°C/min to 155°C and afterwards more slowly by 6 °C/min up to 210° and held there for 2min. Helium was used as carrier gas with a constant linear velocity of 50cm/s [85]. The detector temperature was kept at 250 °C and FAME identification was performed in scan mode. Quantification was performed by selected ion monitoring (SIM) of the most intense fragments [85].

Cholesteryl ester analysis by ESI-MS/MS

This method is based on the creation of an aerosol from the analytic by the use of solvents. At a high voltage and under high pressure using argon gas, small charged droplets of the analytic were produced. After the solvent evaporates, the droplets decrease in size and disperse. The

ions were collected into the vacuum of the mass spectrometer and heated in order to leave room for the next analytic aerosol. Lipid extraction was performed in the presence of not naturally occurring lipid species as internal standards and the chloroform phase was dried in a vacuum centrifuge and dissolved in 10 mM ammonium acetate in methanol/chloroform (3:1 vol/vol). Samples were analyzed by ESI-MS/MS in positive ion mode after direct flow injection using the analytical setup and data analysis algorithms described previously by Liebisch et al. The triple quadrupole MS or tandem mass spectrometry allows to first determine the mass of the ion, then bring ions together with a gas and determine the mass again. **Preparation:** For lipid extraction, the method of Bligh and Dyer was used [86]. 100µl of a chloroform solution containing 100ng/µl of each cholesteryl heptadecanoate (CE 17:0), cholesteryl behenate (CE 22:0) and [25,26,26,26,27,27,27-D_7]-cholesterol were placed into a glass centrifuge tube and evaporated [87]. For absolute quantification, a defined quantity of free cholesterol as well as different naturally occurring cholesteryl ester species were added [87]. The separated chloroform phase was dried and derivatized by adding 200µl of acetyl chloride/chloroform = 1/5 (v/v) for 60min at room temperature and the derivatization reagents were removed by vacuum centrifugation [87] It has to be considered that acetyl chloride decomposes to hydrochloric acid, which may corrode the vacuum centrifuge [87]. The residues were dissolved in 10 mM ammonium acetate in methanol/chloroform = 3/1 (v/v), resulting in a 200-fold dilution corresponding to the initial plasma volume, and 20µl of this solution were injected and data were acquired for 1.3min [87]. **Mass spectrometry:** Samples were quantified by direct flow injection analysis in an HTS PAL autosampler. A flow gradient was performed with a flow of 55 µl/min for 0.1min followed by 30µl/min for 1.0min and an increase to 250µl/min for 0.2 min [87]. The triple quadrupole mass spectrometer with positive ion source was used in a mode with capillary voltage 3.5kV, cone voltage 50V, collision energy 13eV with a collision gas pressure of 1.0×10^{-3} Torr argon, and quantification was achieved by a combination of selected reaction monitoring (SRM) for FC and precursor ion scanning of m/z 369.3 in positive ion mode specific to CE. SRM analysis for FC quantification includes two transitions: m/z 446.4 > 369.3 (CE 2:0) and m/z 453.4 > 376.3 (D_7-CE 2:0) [87]. Labeled $^{13}C_3$-FC and $^{13}C_3$-CE species were monitored by a fragment ion of m/z 372.3 [87]. Lipid extracts were analyzed by thin layer chromatography as previously described by K. Simons et al. [87]. Samples were separated using silica gel HPTLC plates and a solvent mixture of n-hexane/n-heptane/diethyl ether/acetic acid (63/18.5/18.5/1, v/v). Plates were stained by immersion in a manganese/sulfuric acid reagent, followed by heating.

Quantification was performed with authentic FC and CE standards by an analysis of fluorescence excitation using a CAMAG TLC scanner.

PA, LPA, LPC, PC, plasmalogens, sphingomyelins and ceramides

Lipid extraction was performed according to the method of Bligh and Dyer [86] in the presence of not naturally occurring lipid species [88]. The standards were bought from Sigma and Avanti Polar Lipids. The following standards were added: PC 14:0/14:0, PC 22:0/22:0, LPC 13:0, LPC 19:0, Cer 17:0, PA 32:0,PA 36:2, PA 38:4. For the plasmalogens, internal standards were used which do not belong to the same lipid class: PE 14:0/14:0, PE 20:0/20:0. For SPM analysis, the internal standards of PC were used. LPA and PA were identified by liquid chromatographic separation (LC-MS/MS). The internal standard LPA 17:0 was added [89]. The chloroform phase was dried in a vacuum centrifuge and dissolved in 10 mM ammonium acetate in methanol chloroform (3:1vol/vol) [88]. Phosphorous assay was performed according to Bartlett and Lewis [90]. PE-based plasmalogens were quantified according to the principles described by Zemski Berry and Murphy [91]. For this purpose, fragment ions of m/z 364, 380 and 382 were used for PE (P-16:0), PE (P-18:1) and PE (P-18:0) species, respectively [88]. After the identification of relevant lipid species, selected ion monitoring analysis was performed [88].

2.4.2 ELISA

Parameter	Reference range UKR	Source
Adiponectin	2-13.9 µg/ml	Syn Lab
Leptin	3.7-11.1 ng/ml	Labor-Stein[1]
Omentin	2.6-27.4 ng/ml	[92]
Ghrelin (EIA)	200-600 ng/ml	[93]
FGF-23	26-110 U/l	labor-limbach[2]
Apo B-48	3.53-7.39 ng/ml	UKR
SuPar	1.04–4.01 ng/mL	[94]

Figure 14: Reference range of the parameters measured by ELISA.
[1]http://www.labor-stein.de/service/untersuchungsprogramm/?tx_laboratoryeditor_pi1[s_uid]=46161
[2]www.labor-limbach.de

Background: ELISA kits are used for the quantitative determination of the substance examined from EDTA plasma, serum or lithium-heparin plasma. The ELISAs used were simplified double monoclonal antibody “sandwich assays” (Figure 15).

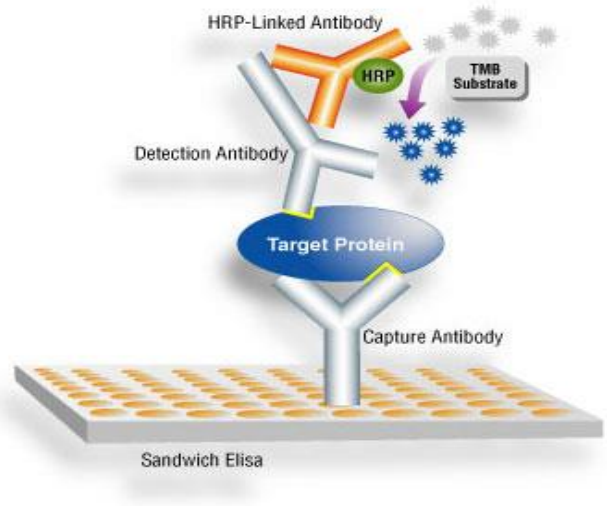


Figure 15: **ELISA sandwich model**, high capture antibody, http://proteomics.case.edu/proteomics/elisa.html (8).

The first layer of the sandwich consisted of a surplus of “capture” antibodies (in our case from mice), which captured the antigen and immobilized it. The capture antibody is fixed to a solid phase and so the antigen is trapped to a solid phase [95]. The second layer consisted of the captured antigen. The biotinylated detection antibody was added and a complex with the antigen was formed. Biotin was used as an amplificator. We used a secondary antibody, streptavidin, which was linked covalently with the enzyme (horseradish) and detected the detection antibody by its high affinity to biotin. After all loose antibodies were washed out, the fixed antibodies were brought together with the enzyme-specific substrate, in this case TMB. The substrate was converted after the enzyme-linked antibody (HRP-linked antibody) was brought together with the substrate (TMB). The subsequent reaction produced a color change. This detectable signal was measured on a Tecan sunrise photometer. The amount of fluorescence corresponds to the amount of antigens.

ELISA: Procedure

As a preparation, the washing solution was diluted ten-fold in distilled water. A serial solution was made with the standard solution and solution buffer. The first concentration produced was 64ng/ml, which was the source for the next solution, until the concentration was 2ng/ml. From these standards, a calibration curve was created. The quality controls were mixed with

the volume of dilution buffer provided. The biotin-labeled antibody concentrate was diluted according to the instructions by mixing it with its diluent. The patients' samples were diluted as recommended in the kits instructions. The standards, quality controls, dilution buffer were transferred to the microplate wells pre-coated with antibodies. The plate was incubated at 37°C for the prescribed time (1 hour for SuPar and leptin, 2 hours for apoB-48 and omentin). After incubation and washing (to remove the unbound material), biotin-labeled polyclonal detection antibodies were added and incubated at 37°C with the captured samples for 30 minutes. After another washing, streptavidin-HRP conjugate was added. After 30 minutes and the last washing step, the remaining conjugate was allowed to react with the substrate solution (TMB). The plate was covered with aluminum foil and incubated for 10 (for SuPar 20) minutes. The reaction was stopped by adding acidic solution. Afterwards the absorbance of the resulting yellow product was measured spectrophotometrically at 450 nm with a Tecan sunrise photometer. The absorbance was proportional to the concentration of the substrate. A standard curve was constructed by plotting absorbance values against the concentration of standard solutions and unkown concentrations of substrates.

2.4.3 EIA

In contrast to the ELISAs applied, with EIA all components were in solution [95]. In our study, a double-antibody sandwich technique was used. The plate was coated with monoclonal mouse antibodies specific to the C-terminal part of human ghrelin. The sandwich was formed together with acetylcholinesterase (AChE) – a Fab' conjugate which recognizes the N-terminal part of des-acylated ghrelin. The concentration of human des-acylated ghrelin was then determined by measuring the enzymatic activity of the immobilized AChE using Ellman's reagent. The AChE tracer reacts to the Ellman's reagent by forming a yellow compound. **Specific description of the EIA used:** In preparation, the EIA buffer was mixed with 50ml distilled water. The standard solution was mixed with 1ml distilled water and after 5 minutes was mixed by inversion. The standard was mixed 7 times with 500 µl buffer, so that a serial dilution was made from 250 pg/ml to 1.96 pg/ml. The quality control was mixed with 1ml distilled water. 10 ml EIA buffer was mixed with anti-des-acylated-ghrelin-AChE tracer. The 1 ml wash buffer was mixed with 400ml distilled water and 200 µL and mixed by magnetic stirrer. Ellman's reagent was mixed with 49ml distilled water and 1ml wash buffer. The plate was prepared according to the instructions.

2.4.4 Clinical chemistry

Parameter	Assay manufacturer	Reference range
Creatinine	Siemens	<1.1 mg /dl
Glucose	Siemens	100 mg/dl
CRP	Siemens	< 5 mg /l
Cholesterol	Siemens	<200 mg/dl
TAG	Siemens	20-200 mg/dl
HDL	Siemens	35-55 mg/dl
LDL	Siemens	<150 mg /dl
Apo A-1	Siemens	115-190 mg/dl
ApoB-100	Siemens	70-160 mg/dl

Table 4: Laboratory internal reference values 12.07.2011

Creatinine, glucose, CRP cholesterol, triglyceride, HDL, LDL were automatically analyzed by ADVIA 1800 (Bayer Siemens)**.** ADVIA is an automatic serum collector system which uses photometric tests. Cholesterol and LDL were measured by the enzymatic method and subsequent measurement was performed by Trinder reaction of the hydrogen peroxide formed in the first step. HDL and TAG were also measured by Trinder reaction. But CRP was measured turbidimetrically. Glucose was measured according to the method by Slein. Apolipoproteins AI and B were determined by nephelometric analysis on a Dade Behring BN II nephelometer.

.

2.4.5 Hematology

For blood count by EDTA, we used a standard hematology analyzer: Sysmex XE-5000. The principle of flow cytometry is based on measuring energy emitted in the form of fluorescent light caused by electrons falling from an elevated to a lower energetic level. The exact degree of differentiation and characteristic of the stained cell was determined fully automatically by combining the information about fluorescence intensity with size and granularity state, which was measured with scattered light.

2.5 Statistical analysis

For data administration, we created a central database in Microsoft Access. This database was linked to Swisslab, making it possible to directly query the parameters in the Swisslab

archive. Excel and SPSS also allowed making a direct query of the substances in the Access database. Excel and SPSS 19.00 were used to produce the graphics. Most of the variables did not follow a normal distribution, so significances were tested by non-parametric tests. For dichotomous categories, Pearson's chi-squared test was used. For paired scaled variables, the Friedman test was used, for non-paired variables the Kruskal-Wallis test was performed to compare the significances of all three groups. Finally, the Mann-Whitney U test was employed to compare all groups with each other. In diagrams, standard errors and standard deviations were shown. I deliberately decided to exclude values beneath the detection limit. One exception was ghrelin, since there was a very small number of cases. Pictures were also created including cases beneath detection limit. The detection limit of the test was 0.2pg/ml. To elevate the number of cases, all values beneath detection limit were set to 0.2 pg/ml. The leukocyte concentration was corrected by the dilution factor. To calculate the dilution factor, the hemoglobin (Hb) concentration at every instant of time was divided by the hemoglobin concentration at time zero. This was necessary because the Hb concentration showed significant fluctuations over time in the metabolic syndrome group (Friedman, $p=0.13$ control; $p=0.18$ risk; $p=0.006$ metabolic syndrome).

The correlations were created in Microsoft Excel. Nominal statistical significance of a correlation was based on a correlation coefficient of > 0.6.

3 Results

3.1 Subject characteristics

Study subjects were characterized and classified into three groups. This was based upon the "International Diabetes Federation" criteria. The stratification of a total 67 participants revealed a control group of 14 subjects, a risk group of 20 subjects and almost half of the study participants were classified as metabolic syndrome. The subject characteristics in the various groups were as follows: The average age in the control group at the time of the study was 47 ± 8, in the risk group it was 51 ± 13 and in the metabolic syndrome group 56 ± 11. Measurements and the evaluation of the questionnaire showed 2 hypertensive subjects among the 20 risk subjects and 14 hypertensive subjects among the 33 metabolic syndrome subjects. Only the metabolic syndrome group contained patients with type 2 diabetes, which was a criterion for that group. Of the 33 metabolic syndrome subjects, 10 suffered from type 2 diabetes. The waist circumference in the control group was 85 ± 6 cm, in the risk group 96 ± 8 cm and in the metabolic syndrome group 117 ± 14 cm (Table 5).

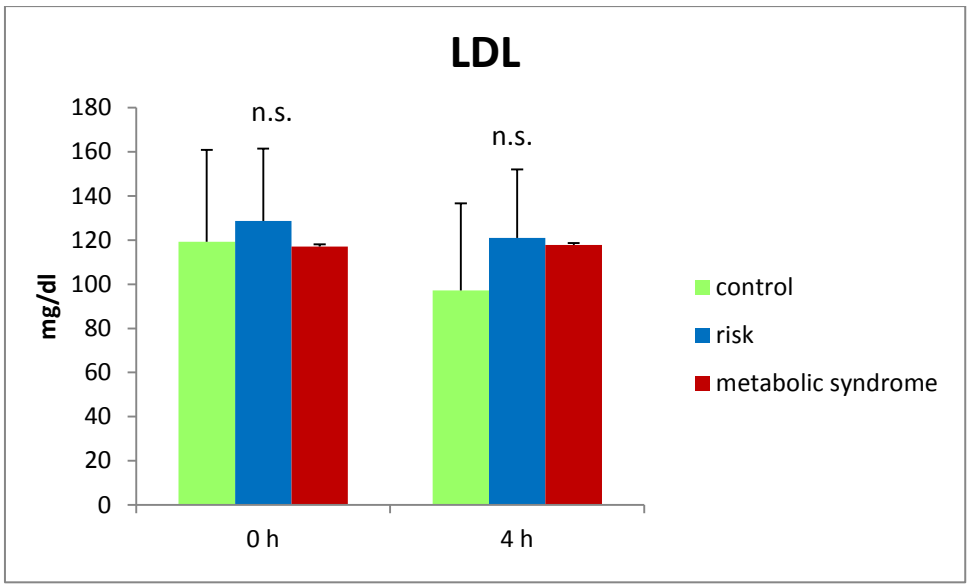
	Control	**Risk**	**Metabolic Syndrome**	**p-Value**
Age	47(±8)	51(±13)	56(±11)	0.03
Waist [cm]	85(±6)	96(±8)	116(±14)	<0.001
BMI	23.4(±2)	26(±3)	33(±7)	<0.001
TAG [mg/dl]	82.5(±32)	121(±46)	202.2(±86)	<0.001
LDL [mg/dl]	119(±42)	129(±34)	115(±33)	0.9
HDL[mg/dl]	66(±16)	56(±16)	42(±12)	<0.001
Non-HDL [mg/dl]	137(±38)	152(±31)	156(±33)	0.2
Cholesterol [mg/dl]	203(±35)	209(±37)	189(±37)	0.6
Glucose [mg/dl]	91(±8)	100(±9)	120(±50)	<0.001
Diabetes frequency	0/14	0/20	10/32	0.002[a]
Hypertonia frequency	0/14	2/20	25/32	<0.001[a]

Table 5: **Characteristics of the groups** (laboratory values are given at time = 0 hours; data is represented as means or frequency, SD is given in brackets. **The calculation of the p-value was performed by Kruskal-Wallis if not marked otherwise by a) where chi-quadrat was used.**

As shown in Table 5, the triglyceride concentration differed significantly between the three groups, with high levels correlating with an increased severity of disease. Subsequently the

cholesterol-transporting lipoproteins (HDL and non-HDL cholesterol, e.g. LDL) were analyzed individually and in relation to the corresponding apolipoproteins (apoB-100, apoB-48, and apo A-I) to derive information about their absolute levels and their atherogenic properties. The results showed that there are significant differences in lipoprotein particle size and distribution between the groups (see Figure 16, Figure 17 and Figure 19).

A



B

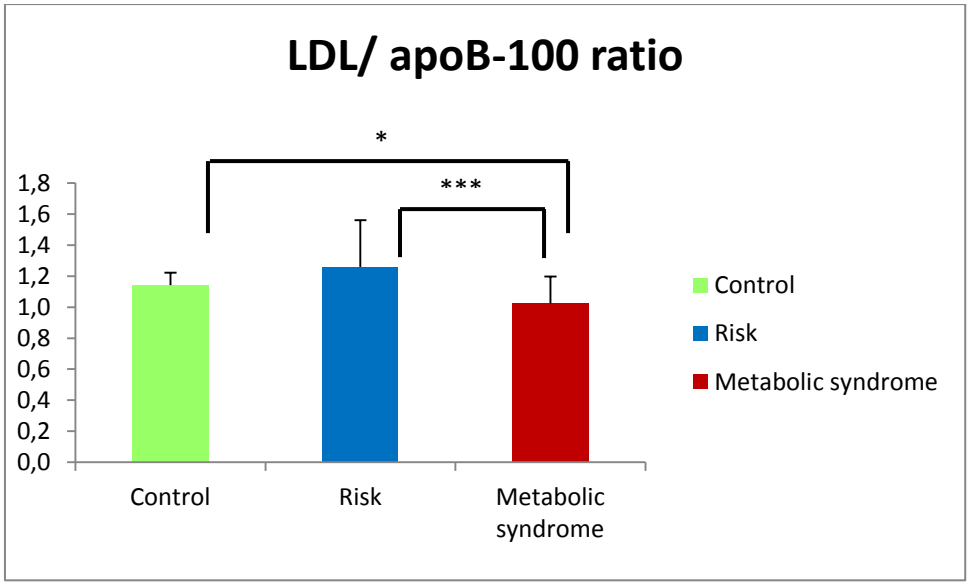


Figure 16: **ApoB-100 containing lipoproteins**. The data is represented as means ± 1 SD, distribution between the groups was compared using the Kruskal-Wallis and Mann-Whitney U tests. Changes over time were tested by Friedman, $p<0.05$ = *; <0.01=**; <0.001=***. A) Comparison of LDL distribution in the three groups at the start of the experiment and at 4 hours B) Comparison of the LDL/apoB-100 ratio in the three groups.

The LDL distribution in fasting and postprandial state in the groups is depicted in Figure 16. The LDL distribution did not differ significantly between the groups (Mann-Whitney U,

p=0.9). However, all of the values were equal and below 130 mg/dl and therefore meet the NCEP guidelines for subjects not having had a coronary heart disease (CHD) event yet [96] (we did not consider diabetes as a CHD-risk equivalent). Also, over time, our results did not show any significant changes in any of the groups (Friedman test, p=0.207, control, n=3; p=0.7, risk, n=7; p=0.59, metabolic syndrome, n=13). The results also showed that the metabolic syndrome group had smaller and therefore potentially more atherogenic LDL particles [97] as demonstrated by calculating the LDL-cholesterol/ApoB-100 ratio, which was significantly higher in the control group than among the metabolic syndrome subjects, yielding a significant p-value of 0.02 (Mann-Whitney U). The control and the risk group did not show significant differences (p=0.13). When comparing the risk and the metabolic syndrome group, p-value was <0.001. However, according to the Friedman test, there were no significant changes over time (Friedman test, p>0.9).

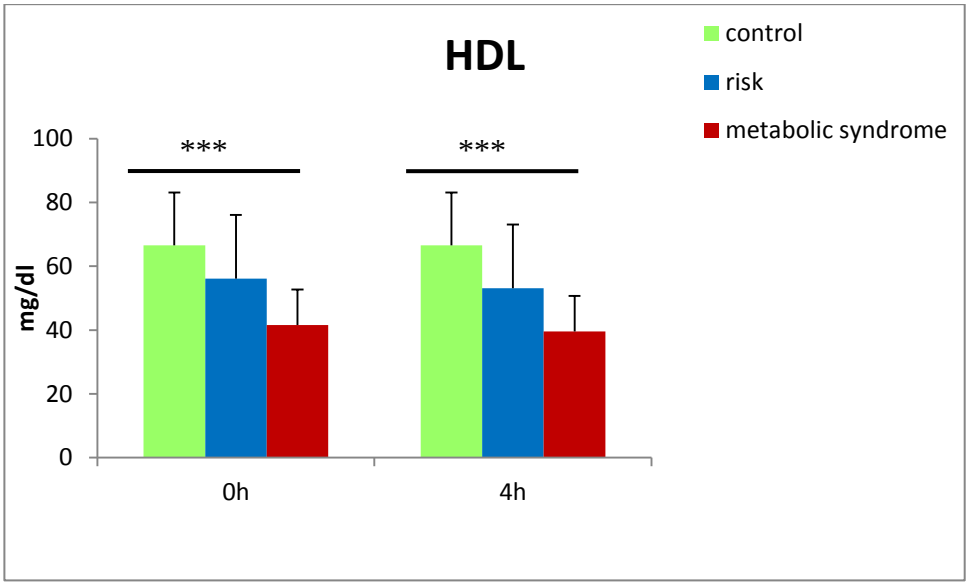
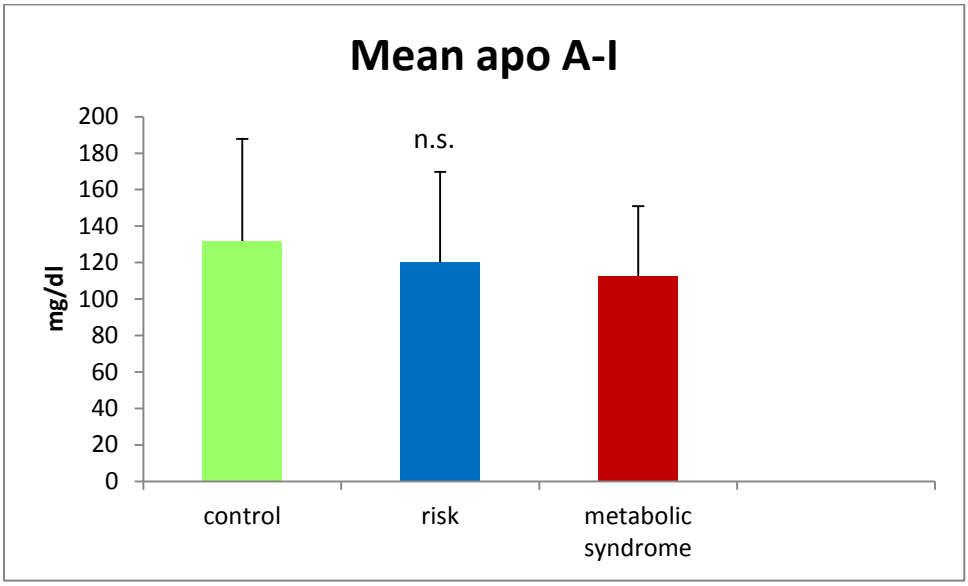


Figure 17: The comparison of the HDL concentration between the groups at t(0) and t(4): n=13 (control), n=19 (risk) and n=32 (metabolic syndrome), p<0.05 = *; <0.01 = **; <0.001 = ***. Data is represented as means ± 1SD.

Since the HDL level was part of the stratification criteria, the baseline values of HDL cholesterol declined from control to risk to metabolic syndrome group as expected. This was confirmed under fasting and postprandial conditions by the non-parametric Mann-Whitney U test (p<0.001). Over time (4 hours) in the postprandial phase, there was no change of the HDL concentration in any of the groups when comparing the HDL plasma concentration under fasting conditions and four hours after the OFTT (Friedman test, p=0.59, metabolic syndrome, n=17; p=0.359, risk, n=7; p=0.354, control, n=3). Next, we looked at HDL's major

apolipoprotein apo A-I to examine particle content in the study groups. “Apo A-I is the major apolipoprotein in HDL particles and has a central role in ‘reverse’ cholesterol transport. Apo A-I can ‘pick up’ excess cholesterol from peripheral cells and transfer it back to the liver in the HDL particles”[98]. Apo A-I also possesses anti-inflammatory, antioxidant effects and anti-atherogenic properties [98].

A



B

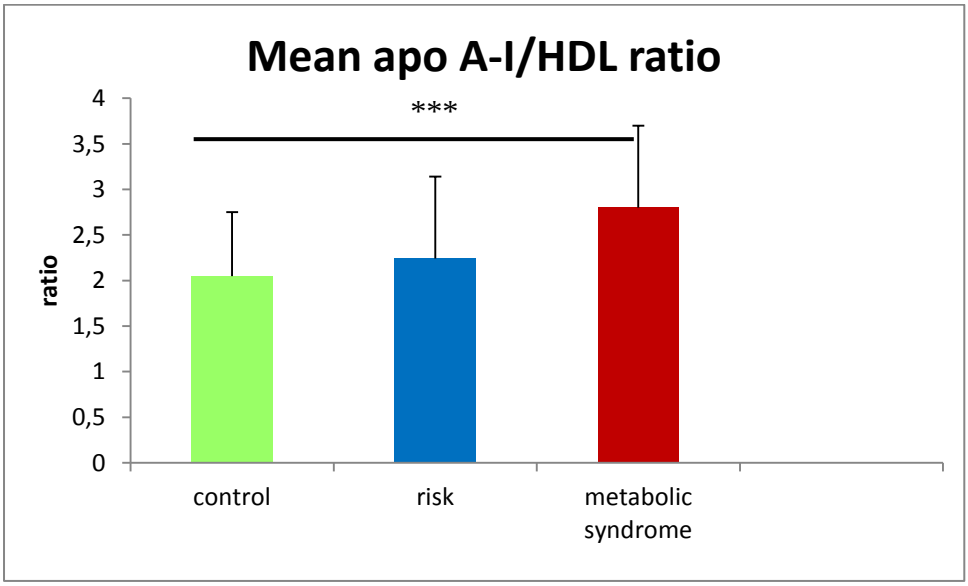


Figure 18: A) **Apo A-I** at t(0), n=12 (control), n=19 (risk), n=32(metabolic syndrome). The differences between the groups were not significant.
B) Comparison of the **apo A-I/HDL ratios** between the groups at time zero t (0) n=12 (control); n=18 (risk) n=31 (metabolic syndrome). Data is represented as means ± 1SD, p<0.05 = *; <0.01 = **; <0.001 = ***. **The apo A-I/HDL ratio was significantly lower in the control group than in the risk group and the metabolic syndrome group at baseline**.

Figure 18A depicts the mean apo A-I concentrations at baseline. Even though the control group showed the highest level of apo A-I, the differences between the groups were not significant (Kruskal-Wallis, p=0.58). Nor was there any significant change in the apo A-I

concentration in any of the groups over time (Friedman, p=0.057, control; p=0.071, risk; p=0.4, metabolic syndrome; supplemental, S 1). Still, the apo A-I/HDL ratio, which is depicted in Figure 18 B, was significantly lower in the control group than in the risk group and the metabolic syndrome group at baseline (Kruskal-Wallis test, p=0.01). Moreover, the results showed that there was an inverse relation between the control, risk and metabolic syndrome groups in terms of HDL and apo A-I levels, indicating an increase in apo A-I containing HDL fractions and an impaired generation of mature, cholesterol-rich HDL in the metabolic syndrome group. This can be deduced from the fact that the premature discoid HDL primarily contains apo A-I [99]. When taking a closer look at the influence of the OFTT on the apo A-I/HDL ratio, the time course showed that during the oral fat tolerance test, there was no significant change in any of the groups (Friedman test, p=0.1, control; p=0.59, risk; p=0.52, metabolic syndrome). Tian et al. found that the apoB-100/apo A-I ratio could reliably and sensitively reflect the HDL subclass profile [100]. The results for the apoB-100/apo A-I ratio are shown in Figure 19.

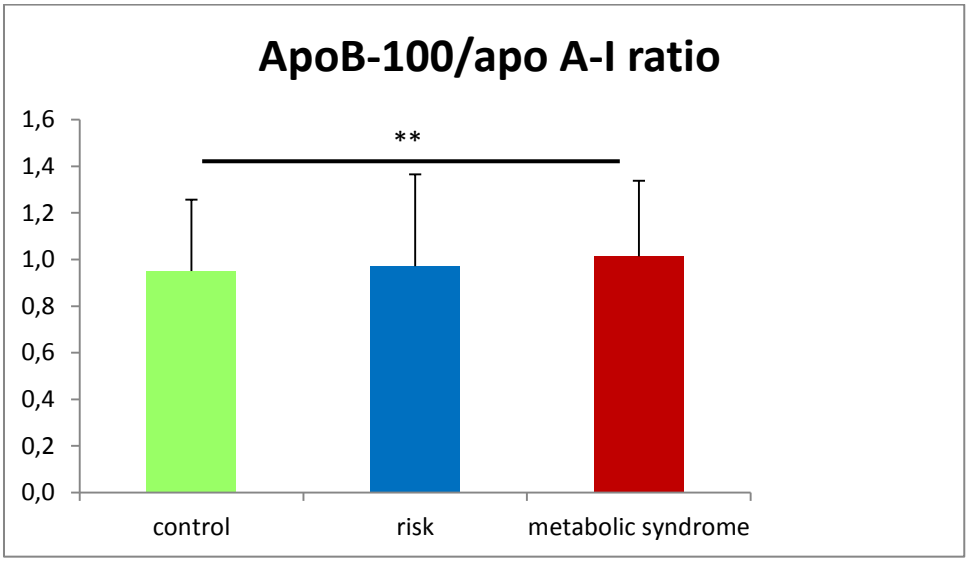


Figure 19: Comparison of the **apo-B100/apo A-I ratio** in the three groups. The data is represented as means ± 1SD, p<0.05 = *; <0.01 = **; <0.001 = ***.

The mean apoB-100/apo A-I ratio was significantly lower in the control group than in the metabolic syndrome group (p=0.002, Kruskal-Wallis). For the purpose of the test, we combined all points in time to obtain a higher number of samples; n=118. The metabolic syndrome group showed a higher apoB-100/apo A-I ratio compared to the other groups. But there was no acute reaction to the OFTT, shown by the fact that there were no significant

changes in any of the groups (Friedman test, p=0.084 control; p=0.32 risk; p=0.9 metabolic syndrome). While apoB-100 is selectively synthesized in hepatocytes and secreted as a constituent of VLDL, apoB-48 originates from intestinal mucosa cells and is secreted as the core apolipoprotein of chylomicrons. Figure 20 represents the apoB-48/apo A-I ratio. Our results demonstrate that the comparison of the means of the apoB-48/apo A-I ratio did not show a significant basal difference between the groups (Kruskal-Wallis test, n=63, p=0.487). To evaluate the effects of the oral fat tolerance test over time, we used the Friedman test (p=0.082, control, n=3; p=0.006, risk, n=6; p=0.001, metabolic syndrome n=12).

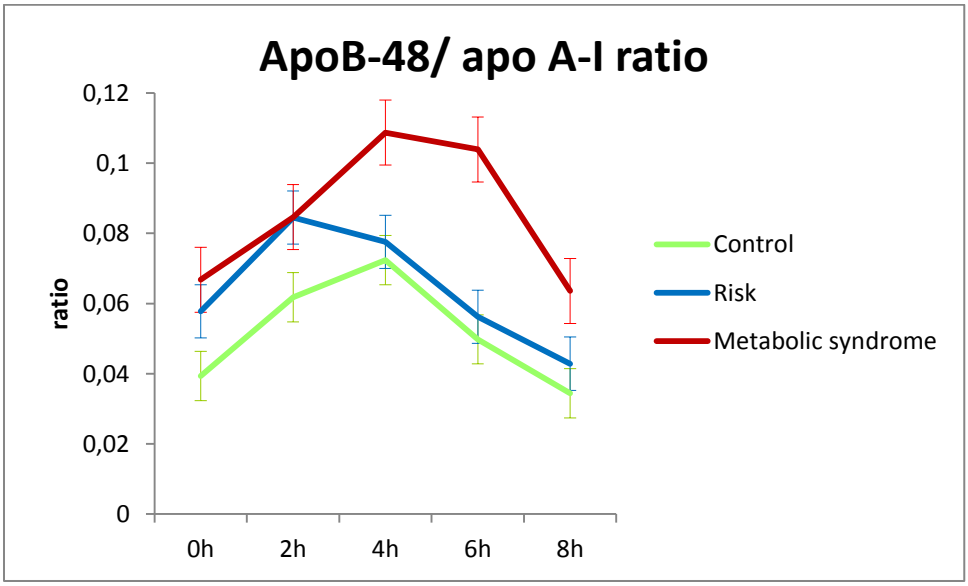


Figure 20: ApoB-48/apo A-I ratio over time. The differences between the groups were not significant. **Over time, the apoB-48/apo A-I ratios showed significant changes in the metabolic syndrome and the risk groups.** The data is represented as means ± 1SD.

In both the risk and the metabolic syndrome groups, an acutely altered metabolism was reflected by an acute response to the drink. Additional data for "ApoB-48" in connection with chylomicrons will be presented in chapter 3.3.2 on plasma lipids.

3.2 Composition of the fat drink

The oral fat tolerance test (OFTT) was carried out as suggested by the Hanefeld group (Dresden) with a minor modification [78]. Table 6 depicts the composition of the oral fat tolerance test according to the formula of Henkel et al.

	Kcal	Protein[g]	Fat[g]	CarboH[g]	PUFA[g]	SUFA[g]	Total FA[g]
250ml/286g milk cream	720	6.2	75	8	2.8	22.6	45.5
100ml/100g fruit juice	53	0.3	0	12	0.2	0	0.1
60g white sugar	243	0	0	60	0	0	0
Total content	1016	6.5 (4.02%)	75 (46.4%)	80 (49.5%)	3	22.6	45.6

Table 6: Contents of the test drink of the oral fat tolerance test.

The total calorie content of the fat drink, based on 250 ml cream plus 100 ml orange juice and 60 g white sugar, was about 1016 kcal, with 66% fat, 32% carbohydrates and 3% protein. To analyze the fatty acid composition of the drink, 10 samples were randomized and measured. The fatty acid composition of the samples was presented and classified according to their degree of saturation (Figure 21A) and according to their chain length (Figure 21B).

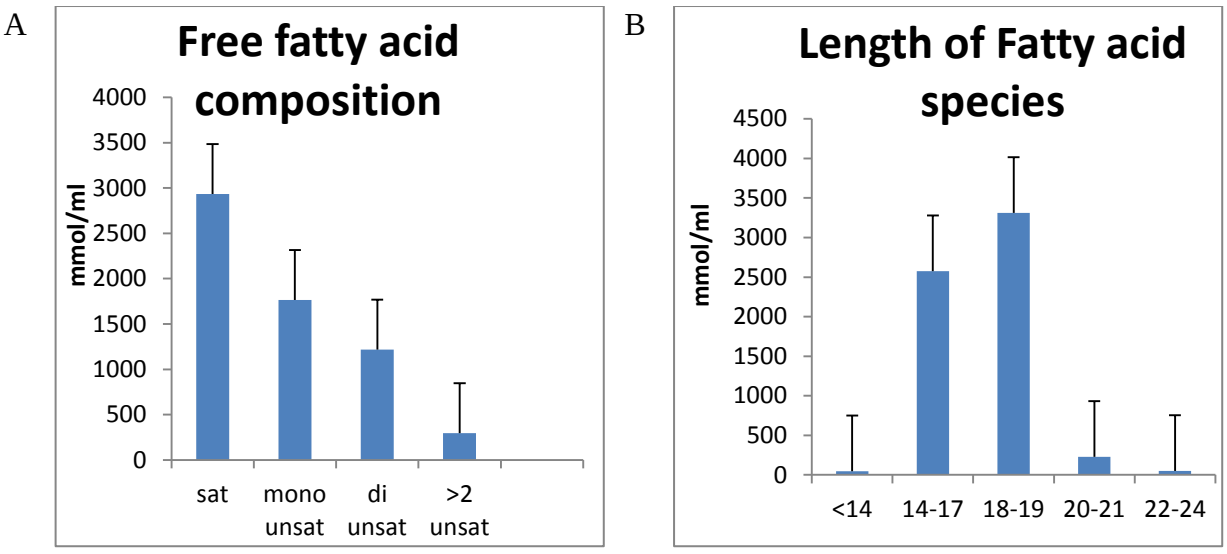


Figure 21: Fatty acid composition of the fat shake. A) Concentration of the FA in the drink, separated according to their degree of saturation B) Concentration of the FA, separated according to number of C atoms. The data is represented as means ± 1SD.

Saturated fatty acids (SUFA) were the predominant type of fatty acids in the drink PUFA/SUFA = 0.13. Among the saturated fatty acids, the most common species is palmitic acid (C16:0), followed by stearic acid (C18:0) and the minor species C13 and C14. (Figure 22) provides a more detailed view of the fatty acid species in the drink.

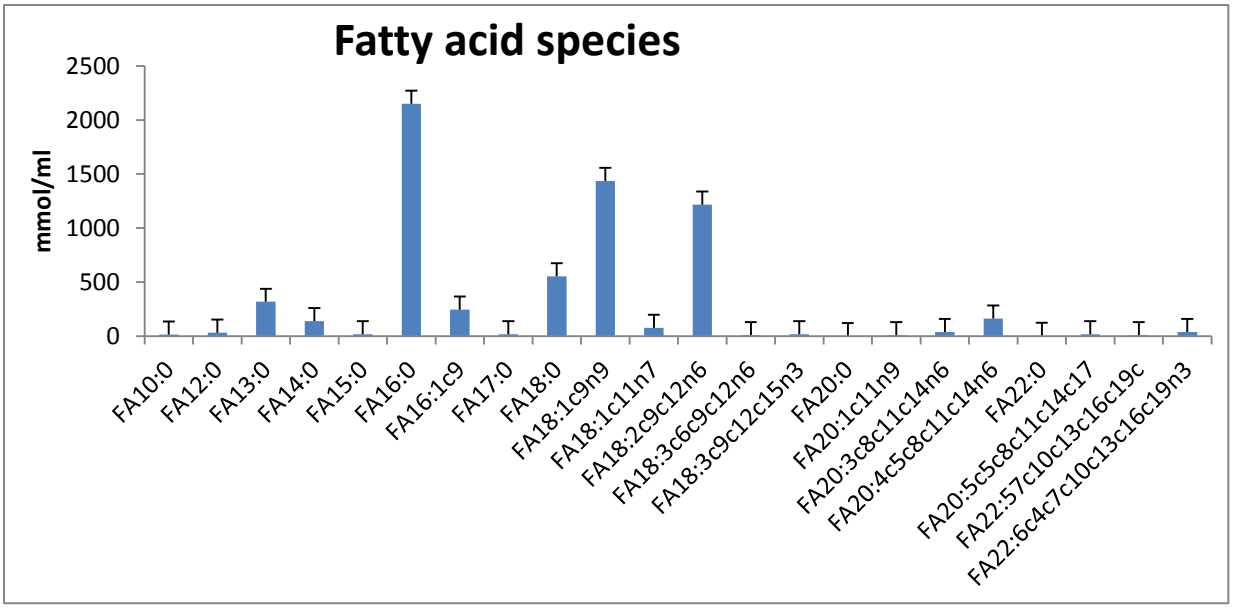


Figure 22: Fatty acid species in the drink. Data is represented as means ± 1SD.

3.3 Plasma lipids

3.3.1 Lipoprotein transport from the intestine: Chylomicron and apoB-48

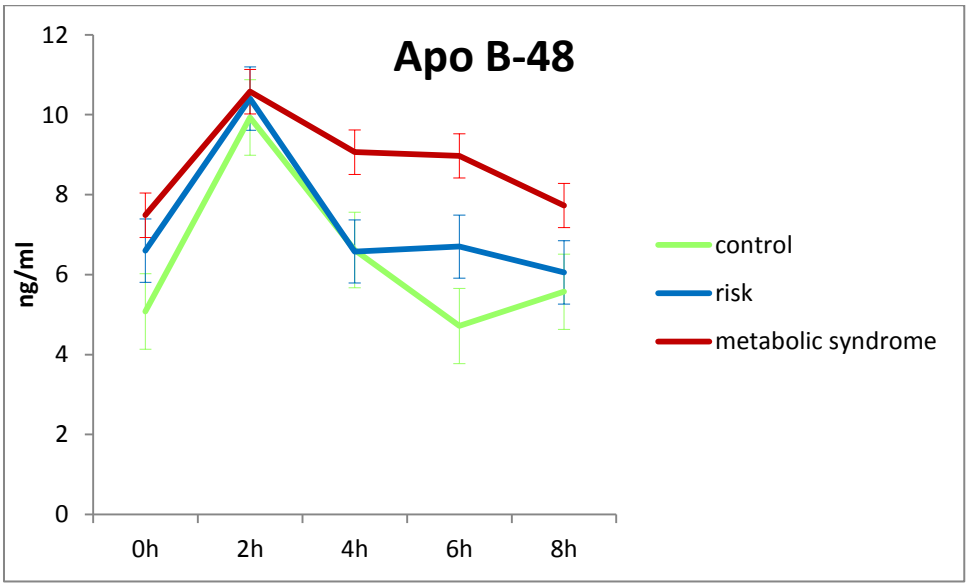


Figure 23: The mean apo B-48 n=14 (control); n=19 (risk); n=33 (metabolic syndrome). The changes over time were significantly different in all groups. Data is represented as means ± 1SD.

The chylomicron-specific apolipoprotein apoB-48 enables the transfer of lipids from the intestinal mucosa to the liver. Figure 23 depicts the apoB-48 concentration in the three groups over time. When only looking at the fasting levels, the difference between the groups was not significant at time zero (Kruskal-Wallis test p=0.607). But a time-independent comparison of

the groups showed significant differences (Kruskal-Wallis test, p=0.02). The calculation was performed considering all points in time between t (0 hours) and t (8 hours). Furthermore, the changes over time were significantly different in all groups (Friedman test; p=0.002, control, n=14; p<0.001 for risk, n=19; and metabolic syndrome, n=33). Moreover, there was a similar peak at t (2 hours) in all groups. But the administration of an OFTT resulted in a lower increase in serum apoB-48 concentration in the metabolic syndrome group compared to an increase in the control group. At t (6 hours), the highest difference between the groups could be observed. The Mann-Whitney U test showed that there was a significant difference between the groups (p=0.026; metabolic syndrome-control). The apoB-48 concentration in healthy controls returned to its basal value after eight hours, whereas the metabolic syndrome group showed no decrease compared to t (4 hours) and did not even reach their basal value after eight hours. The apoB-48/apoB-100 ratio also positively correlates with CAD [101] and is depicted in Figure 24.

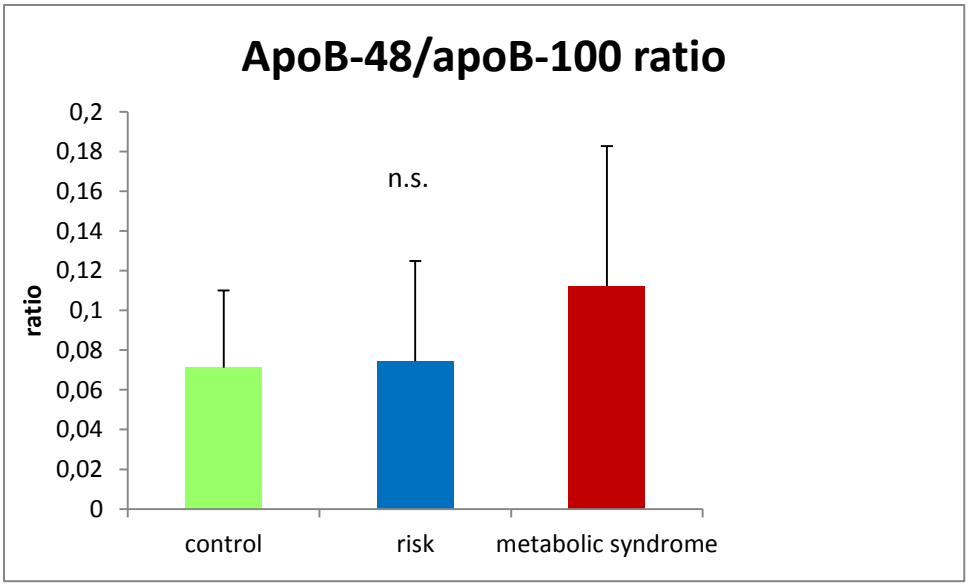


Figure 24: ApoB-48/apoB-100 ratio at time (0 hours), n=45. The differences between the groups were not significant. Data is represented as means ± 1SD.

Our results indicated that even though there was a difference between the groups, this difference was not significant (t0, p=0.246).

3.3.2 Lipoprotein transport from the liver: VLDL and apo-B 100

VLDL carries TAGs from the liver to the periphery. In contrast to the data on apoB-48 (Figure 23), the basal differences in VLDL concentrations between the groups were significant (Figure 25), but there were no changes over time (Friedman test, p=0.717, control, n=4; p=0.96, risk, n=7; p=0.204, metabolic syndrome, n=11).

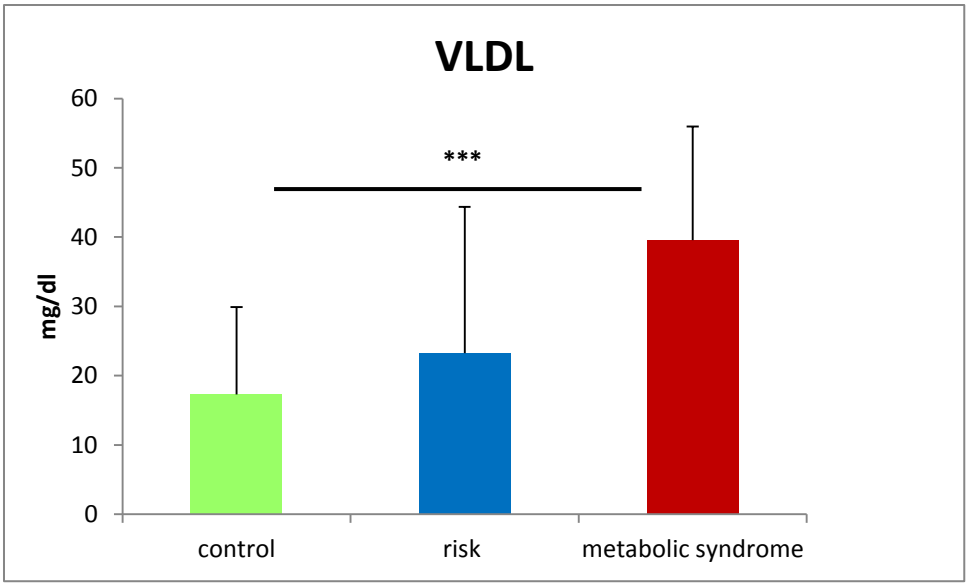


Figure 25: VLDL A) Comparison of the VLDL concentrations between the groups at time zero. Data is represented as means ± 1SE, distribution between the groups was compared using Kruskal-Wallis, p<0.05 = *; <0.01 = **; <0.001 = ***.

The fact that the basal VLDL concentration varied significantly (p<0.001) between the groups was not astonishing, as this is a marker for prolonged postprandial state in steatotic liver disease [102] related to diabesity and metabolic syndrome [103]. However, the control group had a significantly lower basal VLDL level than the metabolic syndrome group. This supported the hypothesis of a prolonged postprandial state of the metabolic syndrome subjects. The OFTT provided additional information related to apoB-100/VLDL metabolism.

3.3.3 Free fatty acids

Major fatty acids in plasma

In order to analyze the free fatty acid composition in metabolic syndrome patients and to find a characteristic reaction in metabolic syndrome subjects following an OFTT, the types of fatty acids in the plasma were measured. The results showed that the major free fatty acids in plasma in all three groups were oleic acid, linoleic acid, arachidonic acid and stearic acid. The major medium-chain fatty acid was tridecanoic acid. When there was a basal difference between the groups, the metabolic syndrome showed the highest values, which will be referred to in more detail below.

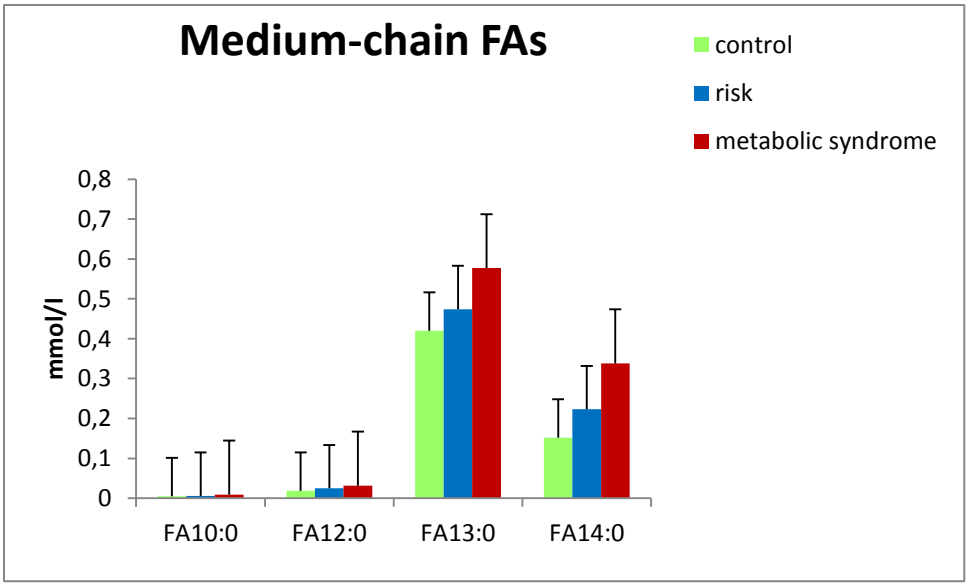


Figure 26: Medium-chain fatty acids at time zero. The differences between the groups were not significant. Data is represented as means ± 1SE.

FA	10:0	12:0	13:0	14:0
p	0.897	0.811	0.853	0.076

The medium-chain fatty acids showed no basal differences between the groups.

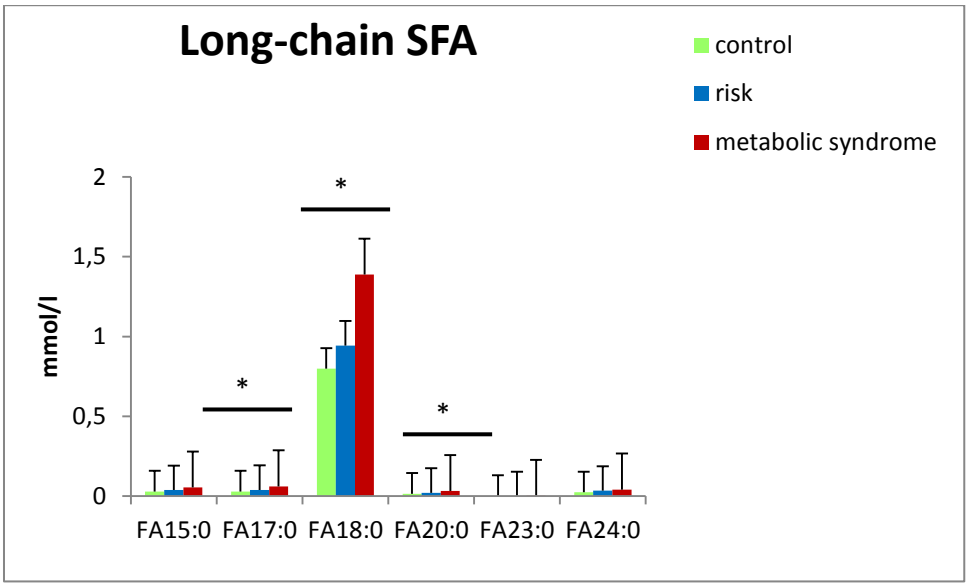


Figure 27: Long-chain fatty acids at time zero, data is represented as means ± 1SE.

FA	15:0	17:0	18:0	20:0	23:0	24:0
p	0.126	0.018	0.048	0.019	0.49	0.13

Comparing the levels of saturated long-chain fatty acids between the groups (Figure 27), margaric acid (17:0), stearic acid (18:0) and arachidic acid (20:0) showed basal differences between the groups. The latter fatty acids are products of the transformation out of palmitic

acid. Due to the pathway of further transformation, the pathway could end up in death or survival. Below, we will refer to those fatty acids and their pathways and products.

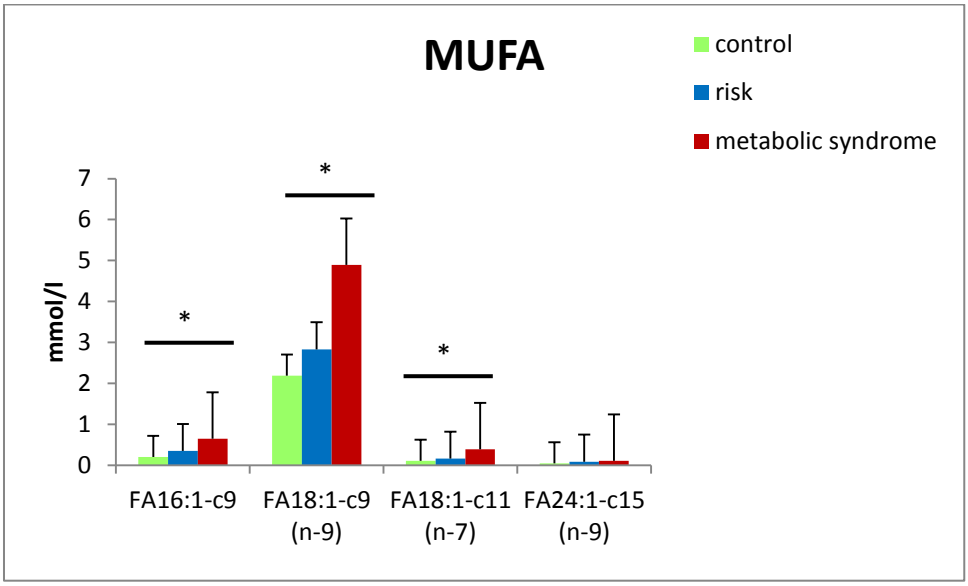


Figure 28: Long-chain monounsaturated fatty acids (MUFAs) at time zero, palmitic acid (p=0.002), oleic acid (p=0.01) nervonic acid (p<0.001), vaccenic acid (p=0.069). Data is represented as means ± 1SE.

Comparing the levels of monounsaturated long-chain fatty acids between the groups (Figure 28), the baseline values of palmitic acid (p=0.002), oleic acid (p=0.01) and nervonic acid (p<0.001) differed significantly between the groups, whereas vaccenic acid did not show different values between the groups (p=0.069). Those types of fatty acids will be referred to in detail below.

FA	16:1- c9	18:1ω9	18:1-c11	24:1ω9
p	0.002	0.01	<0.001	0.069

Comparing the PUFA levels of the three groups, only eicosadienoic acid (20:2 ω 9) and docosatetraenoic (22:4 ω 6) showed differences between the groups at baseline. The omega-3 fatty acids did not show a basal difference between the groups.

FA	18:2ω6	18:3ω6	18:3ω3	20:2ω9	20:4ω6	20:3ω3
p	0.38	0.076	0.244	0.039	0.324	0.499
FA	20:4ω 3	22:1ω9	20:5ω3	22:4ω 6	22:5ω3	22:6ω3
p	0.076	0.43	0.69	0.017	0.336	0.144

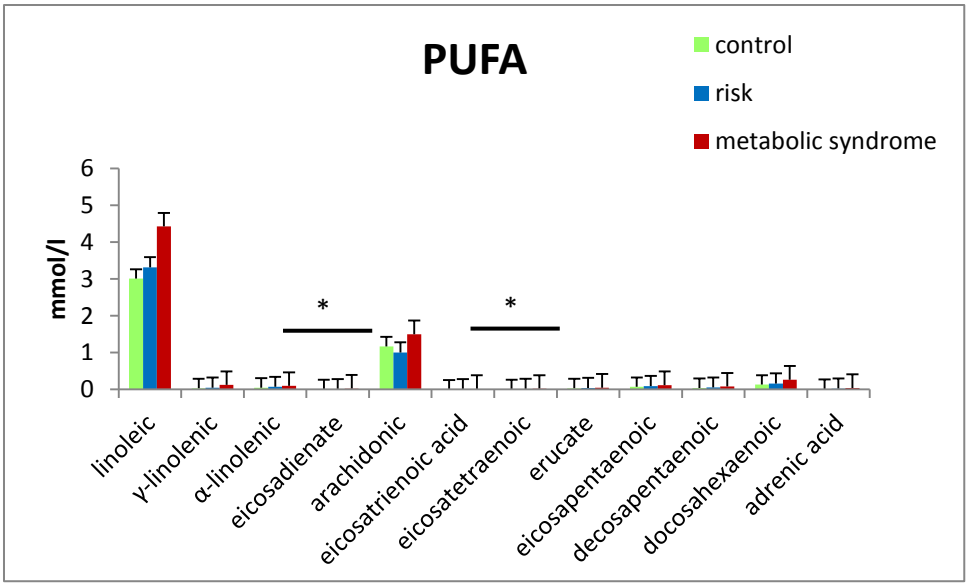


Figure 29: Polyunsaturated fatty acids (PUFAs) at time zero, the names were written out in full (trivial name or name of their salt) in order to avoid a complex systematic nomenclature. Data is represented as means ± 1SE.

This study deals with the development of the pro- and anti-inflammatory fatty acids and their respective precursors, γ-linolenic, arachidonic acid, docosahexaeoic (DAH) and eicosapentaenoic (EPA), over time. Those fatty acid species will be referred to in detail below. When comparing the groups' reaction to the OFTT, we found dissimilarities in the behavior of the following fatty acids which we studied in more detail: palmitoleic acid (16:1c9), eicosapentaenoic acid (EPA, 20:5 ω 3), arachidonic acid (20:4 ω 6), γ-linolenic (18:3 ω 6), eicosanoic acid (20:0), stearic acid (18:0), oleic acid (18:1ω 9) and lignoceric acid (24:0).

Escape pathway in the metabolic syndrome group

Initially, we took a closer look at palmitoleic acid, which has previously been associated with an undesirable adipokine profile [104]. In the case of palmitoleic acid (FA 16:1 (c-9)) (supplemetal, S 2), basal levels differed significantly between the groups ($p=0.002$). Metabolic syndrome subjects showed the highest values. The metabolic syndrome subjects and the risk group showed a significant reaction to the oral fat tolerance test ($p<0.001$, risk; $n=20$; $p<0.001$, metabolic syndrome; $n=32$). Control subjects did not show any significant reaction (Friedman test, $p=0.07$, control; $n=12$).

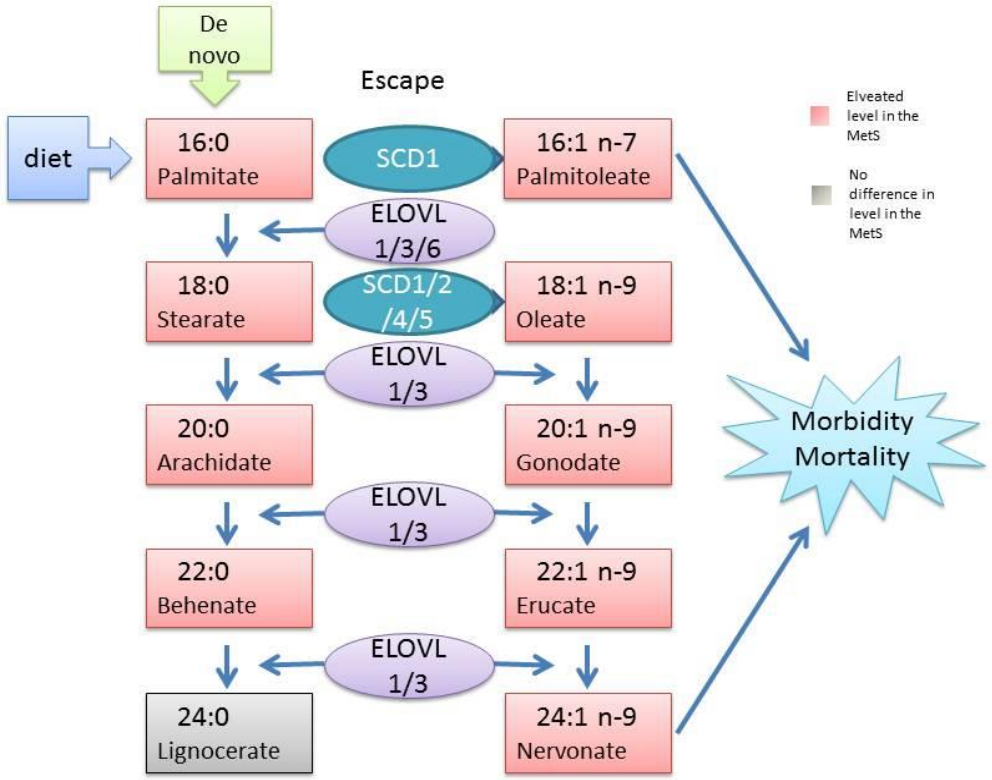


Figure 30: Cell death or survival, based on the diagram by Thomas Kopf.

There are two different synthesis pathways, starting either from fatty acids from the fatty acid palmitate from dietary origin or from de novo synthesis (Figure 30). The level of palmitic acid (C16:0), which was the main type of fatty acid in our oral fat tolerance test, and of products from this fatty acid produced by desaturases and elongases were significantly elevated in the metabolic syndrome group compared to the other groups. The metabolism of this fatty acid could either end in mortality and morbidity ("escape" pathway") or in survival ("normal" pathway) (Figure 30). The product of the "escape" pathway is nervonic acid, which is described in detail below.

In the normal pathway, stearic acid can be produced by palmitic acid. The levels of stearic acid (fatty acid 18:0, supplemental, S 3) showed a significant difference between the groups (Kruskal-Wallis test, $p=0.048$) at baseline. Over time, there was a significant response to the OFTT in all three groups (Friedman test, $p=0.001$, control, $n=12$; $p<0.001$ risk, $n=20$ and $p<0.001$ metabolic syndrome, $n=32$). We were also interested in arachidic acid, also called eicosanoic acid (FA C20:0). Our results are shown in figure S4 in the supplementary. The arachidic or eicosanoic acid (fatty acid 20:0) showed a significant difference between the

groups (Kruskal-Wallis, p=0.019) at baseline. The acute changes in the metabolic syndrome and risk groups were also significant (Friedman test, p=0.004, risk, n=20, risk; p<0.001 metabolic syndrome, n=32), whereas in control subjects, there was no reaction to the oral fat tolerance test (p=0.113, control, n=12). Thus, after the increase in response to the drink, risk and metabolic syndrome subjects showed a decrease after 6 hours. In order to illustrate the escape pathway, oleic acid is depicted in supplemental, S 5. Oleic acid, which can be produced by stearic acid, was also elevated in the metabolic syndrome group compared to the other groups (Kruskal-Wallis, p=0.01). Over time, every group showed an increase, followed by a decrease. However, in the metabolic syndrome group, the increase was delayed (Friedman, p=0.01, control, n=12; P<0.001 risk, n=20, p<0.001 metabolic syndrome, n=32). Moreover, our results (S 6) showed that the level of the ω-9 fatty acid nervonic acid was elevated in the metabolic syndrome group compared to the control group (Mann-Whitney U, p<0.001). Over time, there was no significant change in any of the groups (Friedman, p=0.19, control, n=12; p=0.87, risk, n=20, p=0.17, n=32, metabolic syndrome). Alternatively, nervonic acid could also be produced from lignoceric acid (S 7), which was not significantly elevated in the metabolic syndrome group (Kruskal-Wallis, p=0.35). Over time, a significant response to the oral fat tolerance test only occurred in the metabolic syndrome group, which showed increased levels of lignoceric acid at 4 hours (Friedman, p=0.29, control, n=12; p=0.7, risk, n=20, p=0.01, n=32, metabolic syndrome). From this we can conclude that the elevated level of nervonic acid in metabolic syndrome subjects was produced on the basis of the “escape” pathway, from oleic acid. Nervonic acid is also part of certain types of cytotoxic ceramide species [105]. This ceramide species contributes to the apoptotic effect of lipotoxicity on ß-cells and moreover induces the expression of SCD-1 [105]. It has been published previously that level and activity of Stearoyl-CoA desaturase 1 (SCD-1) – that is the rate-limiting enzyme catalyzing the conversion of saturated to monounsaturated fatty acids – are increased in cardiovascular diseases, insulin resistance and obesity [106,107]. SCD-1 catalyzes the biosynthesis of monounsaturated fatty acids (MUFAs) from saturated fatty acids which are either synthesized de novo or derived from the diet [107]. The endogenously MUFAs synthesized by SCD-1 most likely serve as the main substrates for the synthesis of hepatic TAG and cholesterol esters [107]. The preferred substrates are palmitoyl- and stearoyl-CoA, which are then converted into palmitoleoyl- and oleoyl-CoA respectively [107]. On the other hand, SCD-1 is necessary for the sufficient regulation of the clearance of circulating lipoproteins [107]. A higher ratio of SCD activity can be determined by the following ratios: (16:1n-7/16:0 and 18:1n-9/18:0). SCD activity was determined based on the

products of the desaturation and elongation of palmitoleic acid, the major fatty acid species in the oral fat tolerance test, in order to show that metabolic syndrome subjects are predestined to take the escape pathway, leading to an increased level in nervonic acid. Figure 31 depicts the ratio of the fatty acids 16:1/16:0. The ratio was significantly elevated in subjects with risk for metabolic syndrome and the metabolic syndrome (Kruskal-Wallis, p=0.006). There was no significant difference between the groups concerning the ratio of the fatty acids 18:1/18:0 (Kruskal-Wallis, p=0.16).

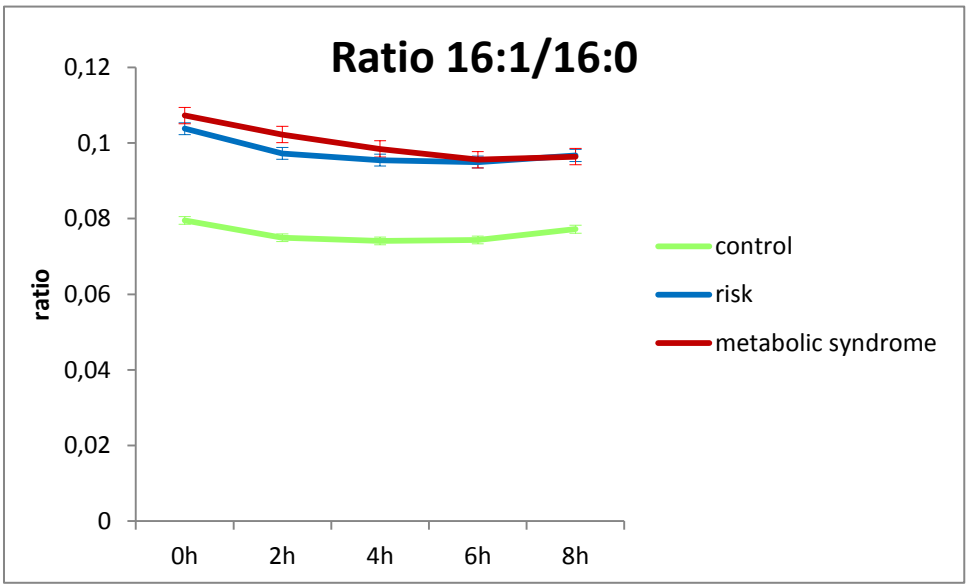


Figure 31: The fatty acid ratio (16:1/16:0), the.concentration differed significantly between the three groups, (Kruskal-Wallis, p=0.006), fatty acid 16:0 p=0.01., data is represented as means ± 1SE.

ω-3/ ω-6 fatty acids

We then looked at eicosapentaenoic acid (EPA) (Figure 32), a protective ω-3 fatty acid [64] and competitive substrate for the enzymes and products of arachidonic acid metabolism. ω-3-derived eicosanoids antagonize the pro-inflammatory effects of ω-6 fatty acids [64]. Both ω-3 and ω-6 fatty acids are ligands/modulators for the nuclear receptors NFκB, PPAR and SREBP-1c, which control various genes responsible for inflammatory signaling and lipid metabolism. ω-3 fatty acids down-regulate inflammatory genes and lipid synthesis and stimulate fatty acid degradation. In addition, the ω-3/ ω-6 PUFA content of cell and organelle membranes, strongly influences cell death and survival [64].

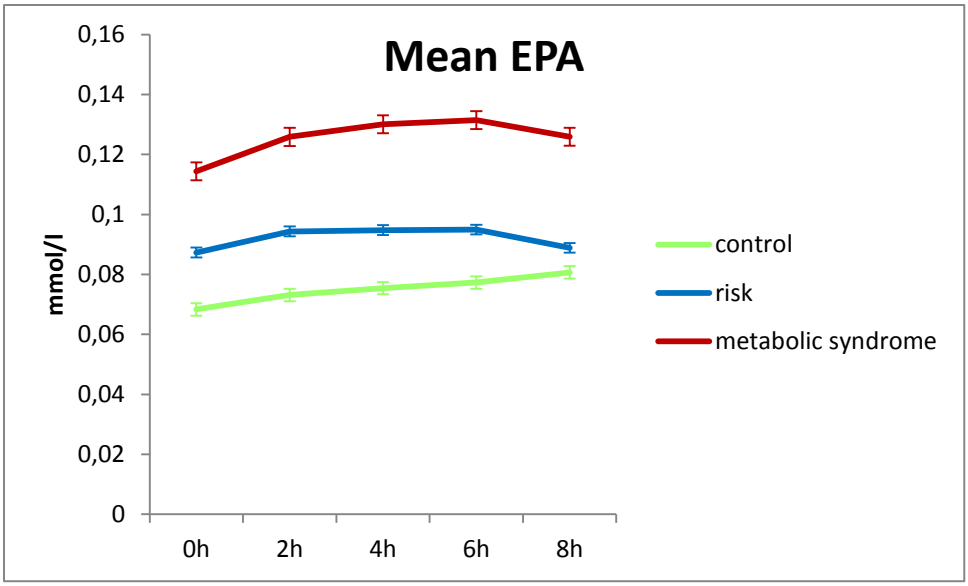


Figure 32: This graph shows the fatty acid 20:5-c5, c8, c11, c14, c17. Significant differences between the groups were calculated using Kruskal-Wallis n=14 control, n=20 (risk); n=33 (metabolic syndrome). Changes over time were evaluated using the Friedman test n=12 (control); n=20 (risk); n=32 (metabolic syndrome). Over time, there was a significant difference in the risk and in the metabolic syndrome group respectively. Data is represented as means ± 1SE.

In terms of eicosapentaenoic acid, called EPA or FA 20:5 with double-bonds at C 5, C 8, C 11, C 14, C 17 there were no significant differences between the groups at time zero (Kruskal-Wallis, p=0.696). Our results showed that over time, there was a significant difference in the risk and in the metabolic syndrome group respectively (Friedman test, p=0.004, risk, n=20; p=0.001, metabolic syndrome; n=32). Both groups showed an increase in EPA serum concentration. The control group did not show any reaction to the oral fat tolerance test (p=0.069, control, n=12).

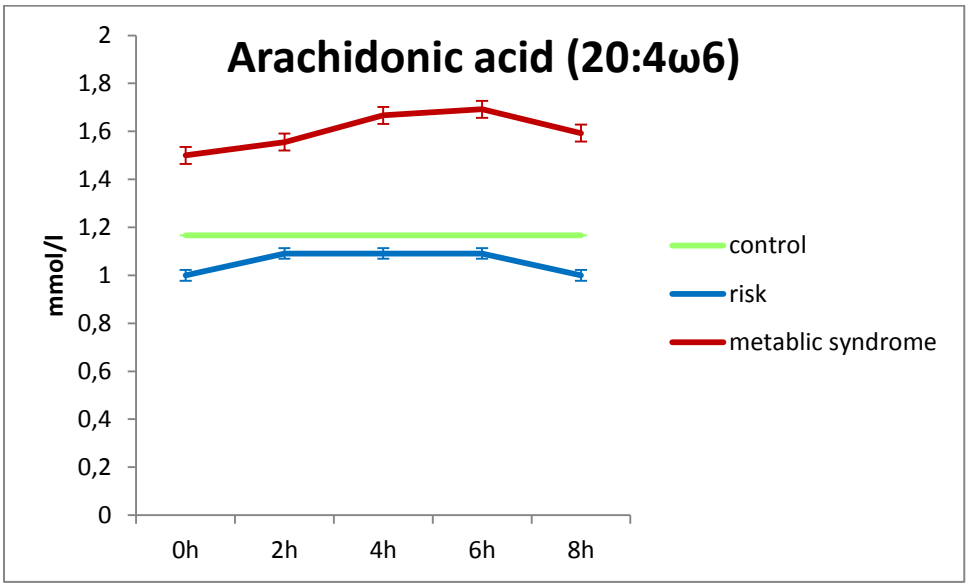


Figure 33: Arachidonic acid (20:4ω6), Kruskal-Wallis test: p=0.324. Differences over time were calculated by Friedman test: p=0.25, control, n=12; p<0.001 risk and metabolic syndrome. Over time, there were differences in risk and metabolic syndrome subjects. Data is represented as means ± 1SE.

The predominant ω-6 fatty acid is arachidonic acid (20:4 ω 6), which is converted to prostaglandins, leukotrienes and other lipoxygenase or cyclooxygenase products [64]. The level of arachidonic acid (20:4 ω 6) showed no significant difference between the groups (Kruskal-Wallis test, p=0.324). Over time, there were differences in risk and metabolic syndrome subjects (Friedman, p<0.001 risk, n=20; p< 0.001, metabolic syndrome, n=32), whereas the control group did not show any reaction to the drink (Friedman, p=0.025, n=12). Another ω-6 fatty acid is γ-linolenic acid (18:3 ω 6). This essential fatty acid was examined in more detail (S 8) because it is the precursor of arachidonic acid (AA). While at the beginning there was no significant difference between the groups (Kruskal-Wallis test, p=0.076), there was a significant difference at two, four and six hours (Kruskal-Wallis test, p=0.03 at 2 hours; p=0.029 at 4 hours, p=0.031 at 6 hours). The difference between the groups disappeared again after eight hours (p=0.11). However, whereas the level of γ-linolenic acid was increased in metabolic syndrome subjects as a response to the fat load, there was no change in the control group and only a slight response in the risk group (Friedman, p=0.6, control, n=12; p=0.01, risk, n=20, p< 0.001, n=32, metabolic syndrome).

3.3.4 Cell death, ceramide with antagonistic effects

What was interesting for us was the ratio of ceramides with antagonistic effects: 24:0 and 24:1 [108]. The former possesses antiapoptotic properties; the latter is responsible for cell death.

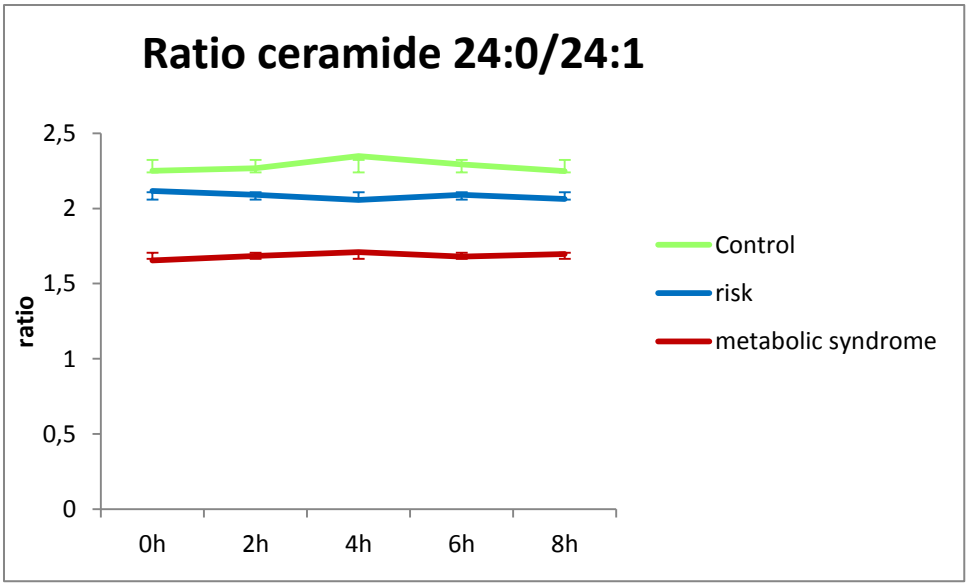


Figure 34: This picture shows the ratio of ceramide 24:0/ceramide 24:1. Groups were compared using Kruskal-Wallis n=14 (control); n=19 (risk); n=32 (metabolic syndrome). There was a significant difference between the groups. Changes over time were evaluated using the Friedman test, n=14, (control) n=18 (risk), n=31(metabolic syndrome). There were no significant differences over time. Data is represented as means ± 1SD.

A negative relationship was found between the ceramide 24:0–24:1-ratio (Figure 34) and the risk of metabolic syndrome (Kruskal-Wallis, p=0.003). The control group showed the highest level of ceramide 24:0 and the lowest level of 24:1 compared to the risk and the metabolic syndrome groups. Over time, there were no significant changes in any of the groups (Friedman, p=0.68, control, n=14; p=0.581, risk, n=18; p=0.626, metabolic syndrome, n=31). Thus, there were no reactions to the oral fat tolerance test in any group.

3.3.5 Signal transduction, Sphingolipids

Sphingomyelin (SPM), which belongs to the class of sphingolipids, is one of the major phospholipids in human cells and plays an important role in signal transduction, particularly with regard to growth and differentiation [109]. It is also a major component of lipid rafts in membranes (cholesterol-/sphingolipid-rich domains) [109]. Previous investigations stated increased sphingomyelin levels in human familial hyperlipidemia, especially in familial hypercholesterolemia and also in animal models of atherosclerosis [110]. Moreover, the SPM concentration in plasma correlates positively with the susceptibility of lipoprotein SPM to sphingomyelinase (SMase) [110].

Schlitt et al. investigated the role of SPM in atherosclerosis by measuring plasma-SPM levels in 1.102 patients with angiographically proven coronary artery disease (CAD) and 444 healthy controls. They found increased plasma levels in CAD subjects [110].

Elevated ceramide production from SPM and ceramide de novo production, both triggered by increased levels of palmitic acid and LPS due to gram-negative bacteria infection, are both involved in the development of chronic states of low-grade inflammation in patients with type 2 diabetes and/or obesity [111]. Figure 35, Figure 36, Figure 37 show the distribution of sphingomyelin species in the three groups control, risk and metabolic syndrome.

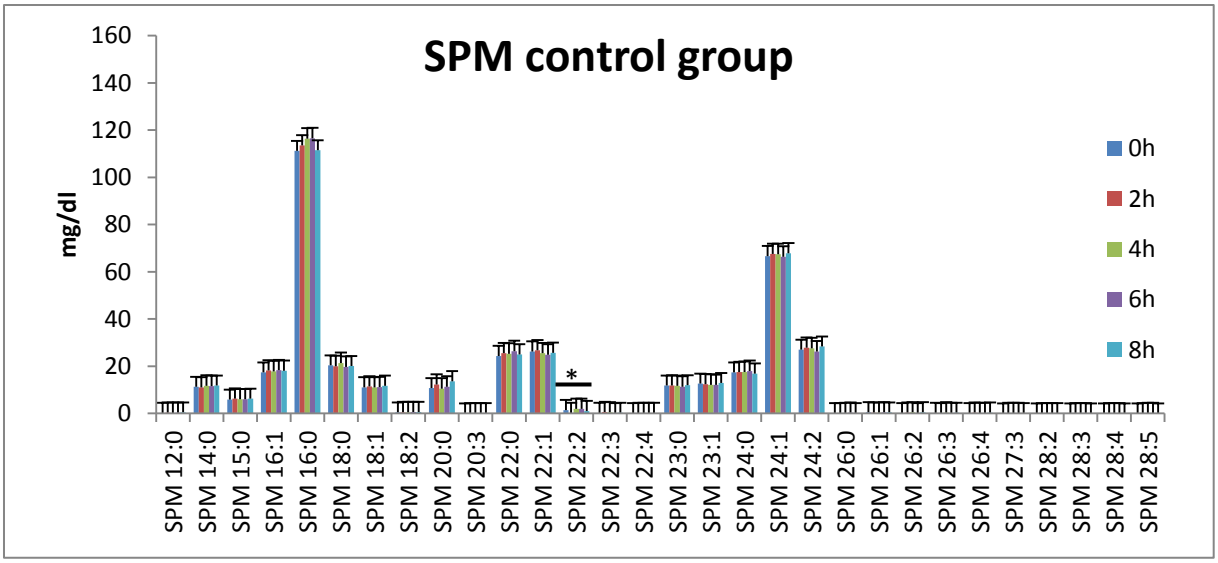


Figure 35: Circulating SPM species concentration in plasma over time in the control group. Data is represented as means ± 1SE.

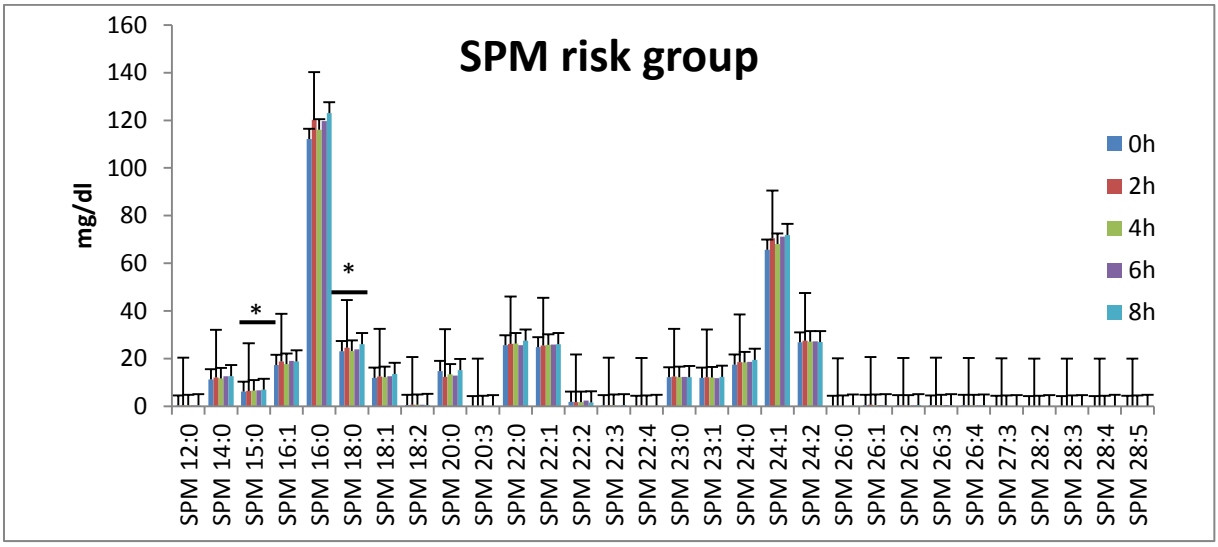


Figure 36: Circulating SPM species concentration in plasma over time in the risk group. Data is represented as means ± 1SE.

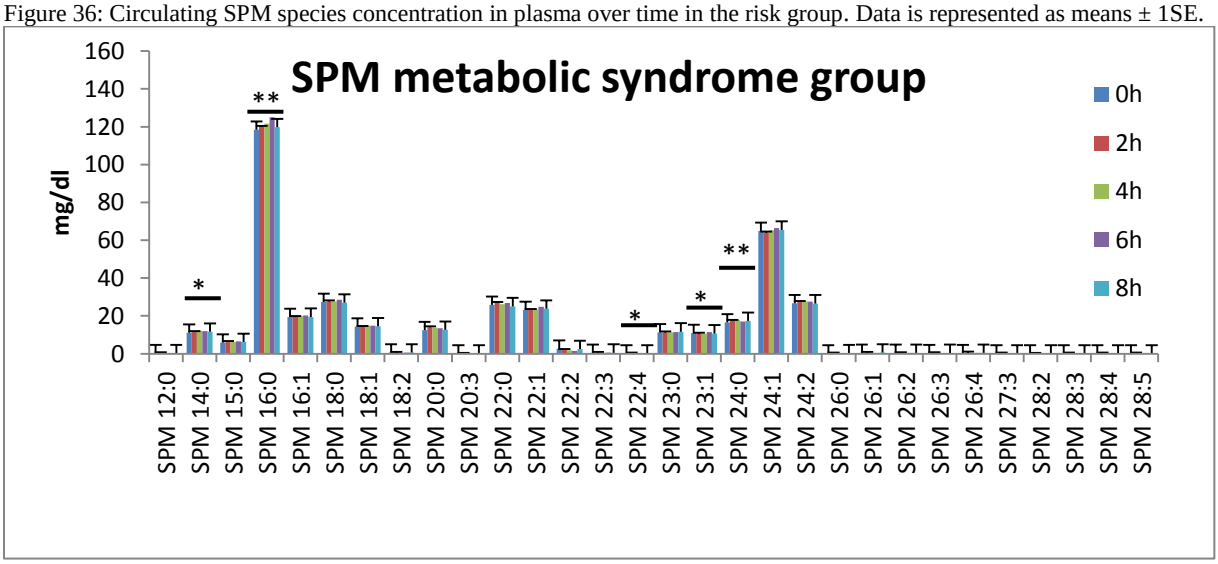


Figure 37: Circulating SPM species concentration in plasma over time in the metabolic syndrome group. Data is represented as means ± 1SE.

Figure 35, Figure 36, Figure 37, show that the most abundant SPM species were SPM 16:0 and 24:1 in all three groups. The sphingomyelin species (SPM 18: 0, SPM 18:1, SPM 22:4), which showed different baseline values between the three groups, are graphically represented in supplemental S 9, S 10, S 11.

SPM species	p-value
SPM 12:0	0.328
SPM 14:0	0.613
SPM 15:0	0.622
SPM 16:0	0.695
SPM 18:0	0.035 *
SPM 20:0	0.143
SPM 22:0	0.679
SPM 23:0	0.195
SPM 24:0	0.377
SPM 26:0	0.538
SPM 16:1	0.266
SPM 18:1	0.041 *
SPM 22:1	0.238
SPM 23:1	0.164
SPM 26:1	0.594
SPM 24:1	0.730
SPM 18:2	0.756
SPM 22:2	0.236
SPM 24:2	0.803
SPM 26:2	0.059
SPM 28:2	0.071
SPM 20:3	0.464
SPM 22:3	0.681
SPM 26:3	0.744
SPM 27:3	0.745
SPM 28:3	0.212
SPM 26:4	0.450
SPM 22:4	0.015 *
SPM 28:4	0.760
SPM 28:5	0.598

Table 7: Comparison of the SPM species between the groups, p-value of Kruskal-Wallis test.

Among the saturated SPMs, only SPM 18:0 showed significantly different baseline levels between the groups S 9. Over time, only the risk group showed a significant change. The peak in the metabolic syndrome group at 6 hours was also remarkable. However, the significant peak that can be seen in the risk group at 4 hours occurs in the metabolic syndrome group later (6h). Among the monounsaturated SPMs, only SPM 18:1 (S 10) showed significantly different baseline levels between the groups. However, the monounsaturated and the saturated

SPM species with 18 C atoms showed a significant difference between the groups. Among the polyunsaturated SPM species, only SPM 22:4 showed a significant difference between the baseline levels of the groups (S 11). This figure shows the peak in the control and the risk groups at 2 hours, whereas the response in the metabolic syndrome group was in delay and showed a peak at 6 hours. The changes over time were not significantly tested by the Friedman test. Next, a detailed description of the sphingomyelin profile over time as a response to the OFTT was carried out. Over time, the following sphingomyelin species showed significant responses to the OFTT. SPM 22:2 showed a significant difference in the control and the metabolic syndrome groups over time (p=0.04 control, p=0.39 risk, p=0.024 metabolic syndrome); SPM 15:0 (p=0.49 control, p=0.013 risk, p=0.24 metabolic syndrome) and SPM 18:0 (p=0.27 control, p=0.004 risk, p=0.244 metabolic syndrome). The latter showed a significant difference in the risk group (S 9). SPM 14:0 (p=0.49 control, p=0.075 risk, p=0.026 metabolic syndrome), SPM 23:1 (p=0.84 control, p=0.40 risk, p=0.016 metabolic syndrome), SPM 24:0 (p=0.72 control, p=0.47 risk, p=0.003 metabolic syndrome), SPM 16:0 (p=0.9 control, p=0.15 risk, p=0.009 metabolic syndrome) only showed a response in the metabolic syndrome group.

SPM	Control (p)	Risk (p)	Metabolic syndrome (p)
SPM 12:0	0.37	0.28	0.21
SPM 16:1	0.97	0.149	0.39
SPM 18:2	0.96	0.65	0.48
SPM 18:1	0.4	0.25	0.28
SPM 20:3	0.9	0.35	0.08
SPM 20:0	0.7	0.08	0.5
SPM 22:3	0.08	0.82	0.9
SPM 22:4	0.29	0.82	0.12
SPM 22:1	0.62	0.89	0.19
SPM 22:0	0.66	0.22	0.19
SPM 23:0	0.21	0.92	0.86
SPM 24:2	0.7	0.8	0.2
SPM 24:1	0.76	0.09	0.34
SPM 26:4	0.35	0.78	0.98
SPM 26:3	0.45	0.43	0.6
SPM 26:2	0.08	0.5	0.31
SPM 26:1	0.6	0.5	0.4
SPM 26:0	0.4	0.9	0.6
SPM 27:3	0.9	0.9	0.72
SPM 28:5	0.4	0.054	0.7
SPM 28:4	0.9	0.57	0.8
SPM 28:3	0.67	0.58	0.59
SPM 28:2	0.51	0.09	0.12

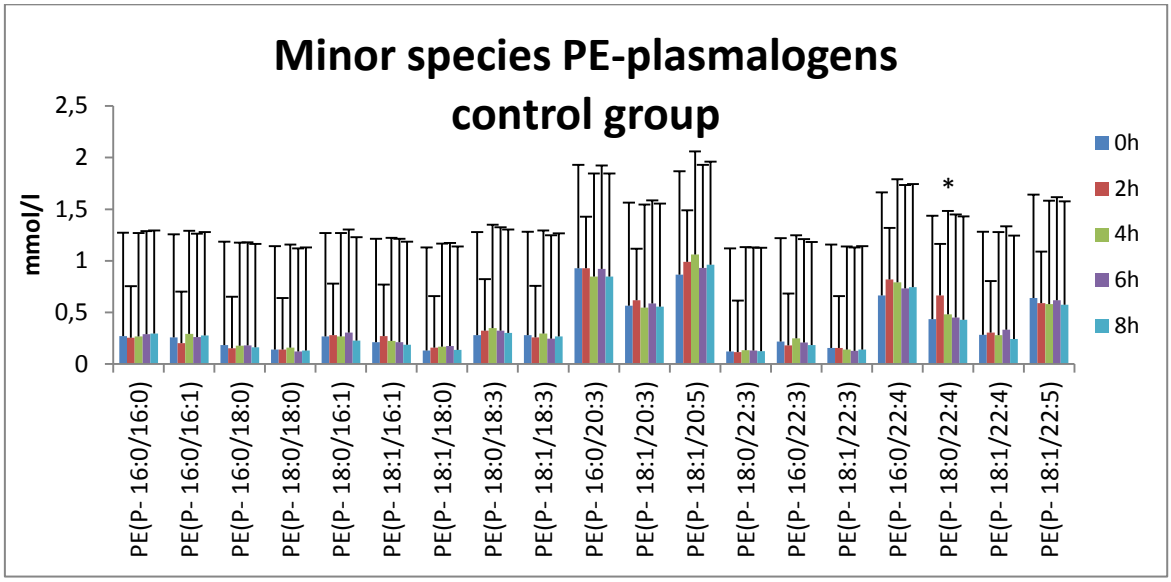
Table 8: p-values that were not significant. Comparison of the SPM species over time, p-value calculated by Friedman test

Those SPM species which could be relevant as biomarkers to identify metabolic syndrome subjects, due to their different responses to the OFTT compared to the other groups, are separately shown in graphics in the supplemental. Those were: SPM 14:0 (S 12), SPM 16:0 (S 13), SPM 22:2 (S 14), SPM 23:1 (S 15) and SPM 24:0 (S 16). All of these species either showed a response only in the metabolic syndrome group but not in the other groups, or a different response in the three groups. The SPM species 16:0 showed no baseline differences between the groups (Kruskal-Wallis test p=0.69). The trend showed a progressively higher baseline level from the control to the risk to the metabolic syndrome groups. A significant increase at 6 hours could only be observed in the metabolic syndrome group. Also for SPM 22:2, there was no significant difference between the groups when comparing the baseline levels. The metabolic syndrome showed a trend towards higher levels. A decrease as a response to the drink was observed in all three groups, but in the risk group the changes over time were not significant. In all cases, the metabolic syndrome showed a delayed decrease and thereafter delayed normalization. The SPM species 23:1 seemed to be elevated in the control group, even though not significantly. In the metabolic syndrome group, there was a significant increase 6 hours after the fat load. The other groups showed no response to the OFTT. 24:0 belongs to the most protective SPM species and it showed decreased levels in the metabolic syndrome group. The metabolic syndrome group reacted to the OFTT by increasing its level. This increase could be interpreted as a protective response to an additional fat excess.

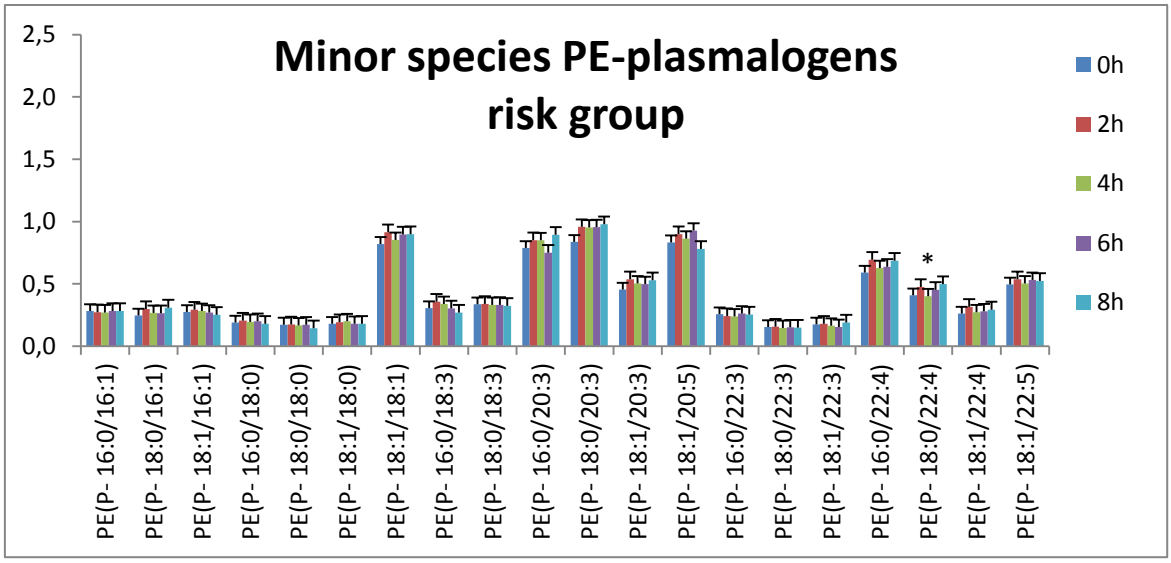
3.3.6 Antioxidants: Plasmalogens

Plasmalogens are phospholipids carrying fatty acid-ether bonds in sn-1 position, with C16:0, 18:0 and 18:1 fatty acids and fatty acid esters in sn-2 position [62]. The fatty acid composition of plasma phospholipids reflects the ingested fatty acids, but is also influenced by various factors. The polyunsaturated fatty acids in sn-2 position are precursors for ω-3- or ω-6-based prostaglandins which are antagonists in inflammation and thrombosis [88]. The response of the plasmalogens to the OFTT in the three groups is shown in (Figure 38, Figure 39, Figure 40). The plasmalogen species were separated into those which occur rarely, commonly and frequently and their dynamics in the respective groups were depicted over time in order to give an overview.

A



B



C

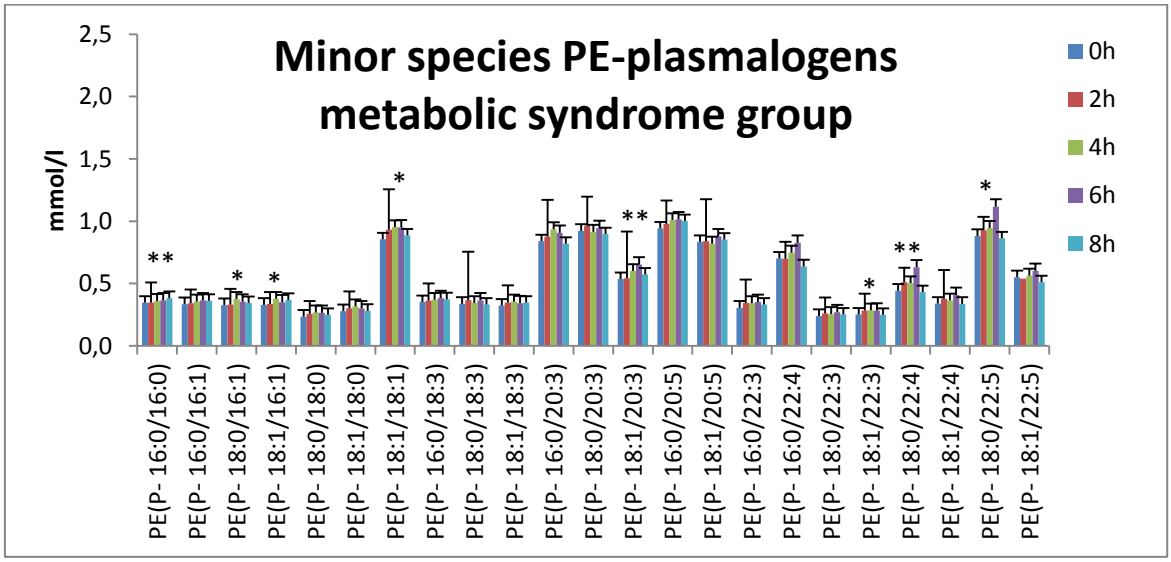
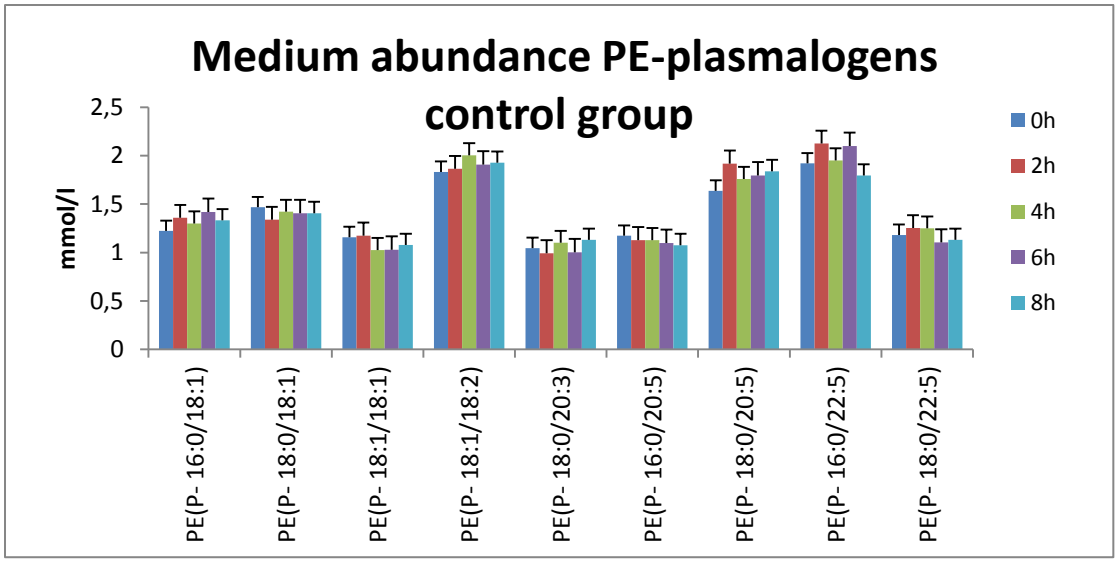
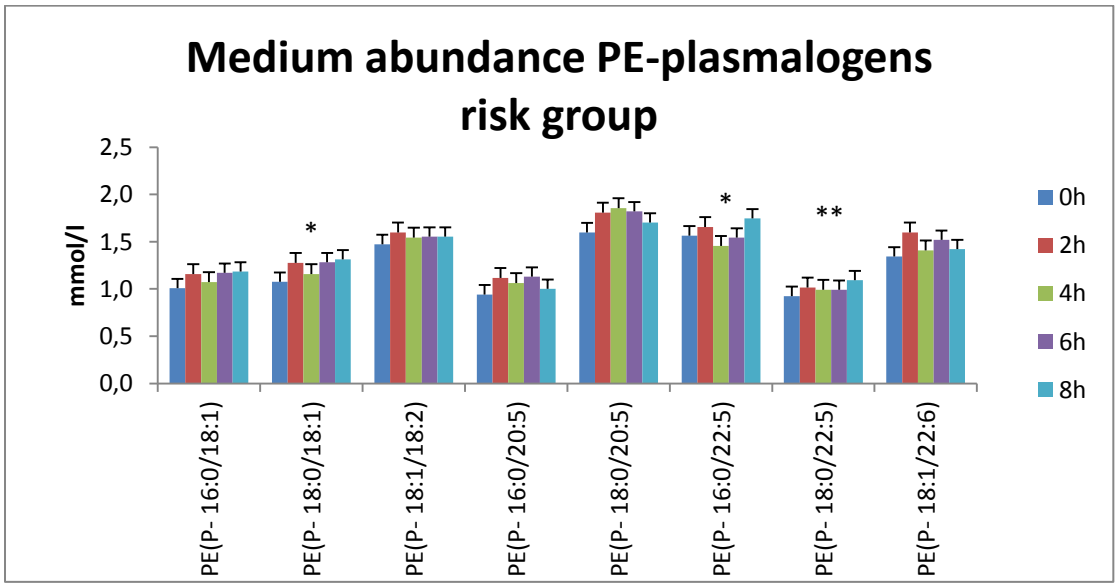


Figure 38: Minor abundance plasmalogens, data is represented as means ± 1SE.

A



B



C

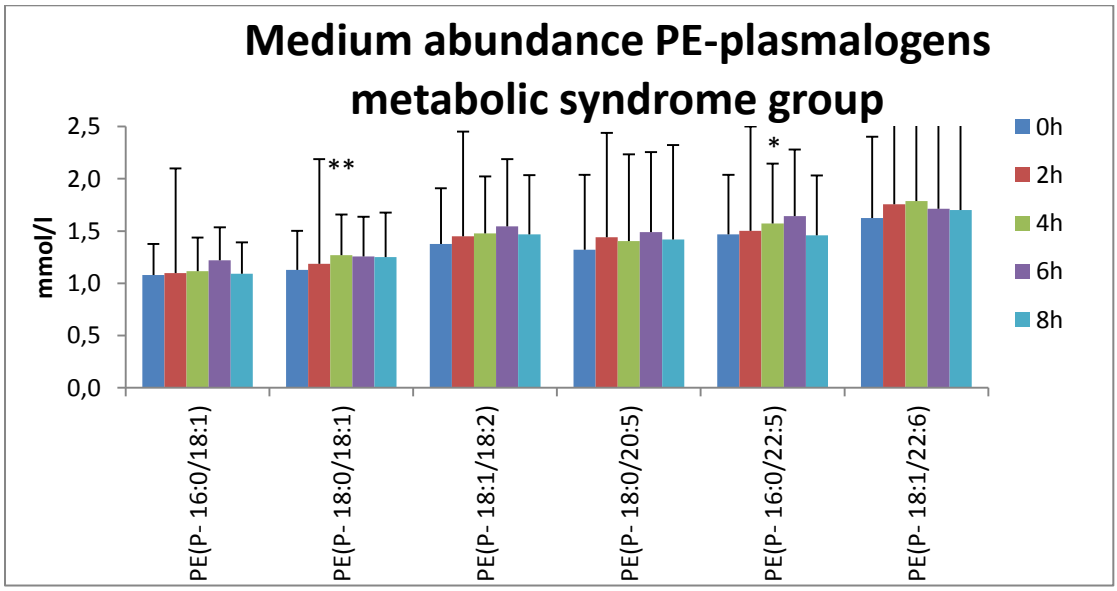
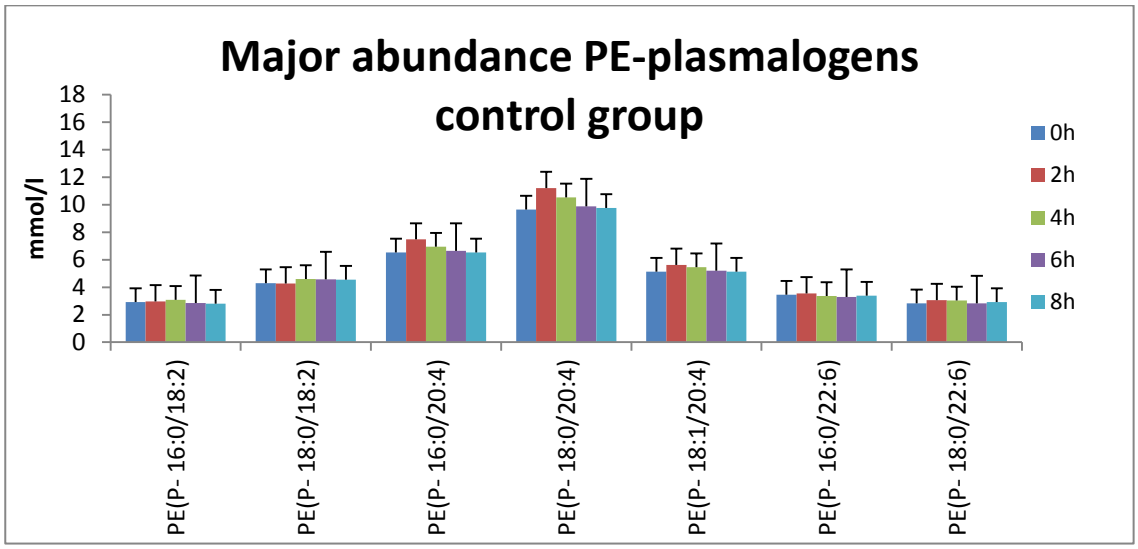
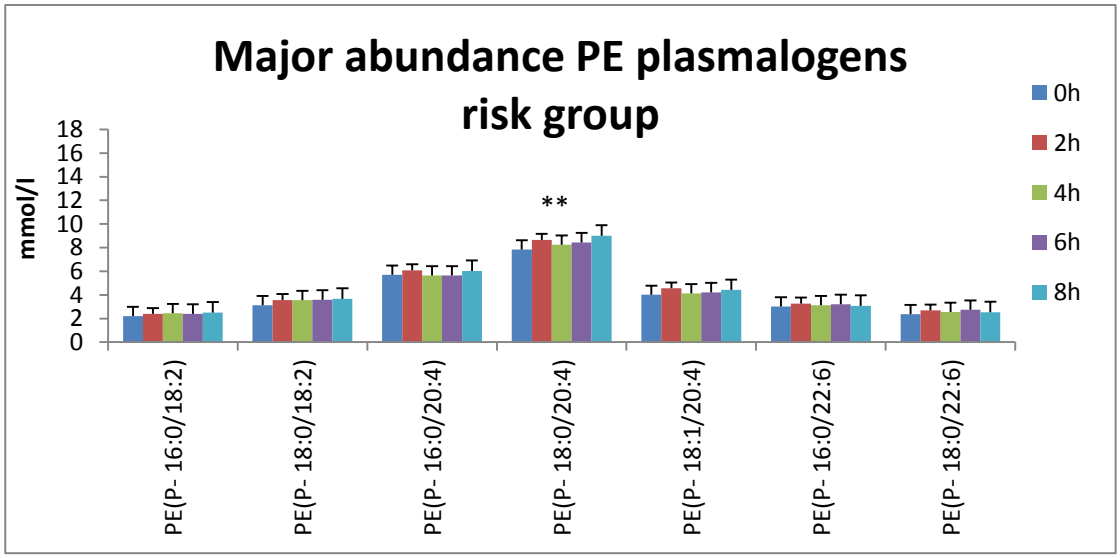


Figure 39: Medium abundance plasmalogens, data is represented as means ± 1SE.

A



B



C

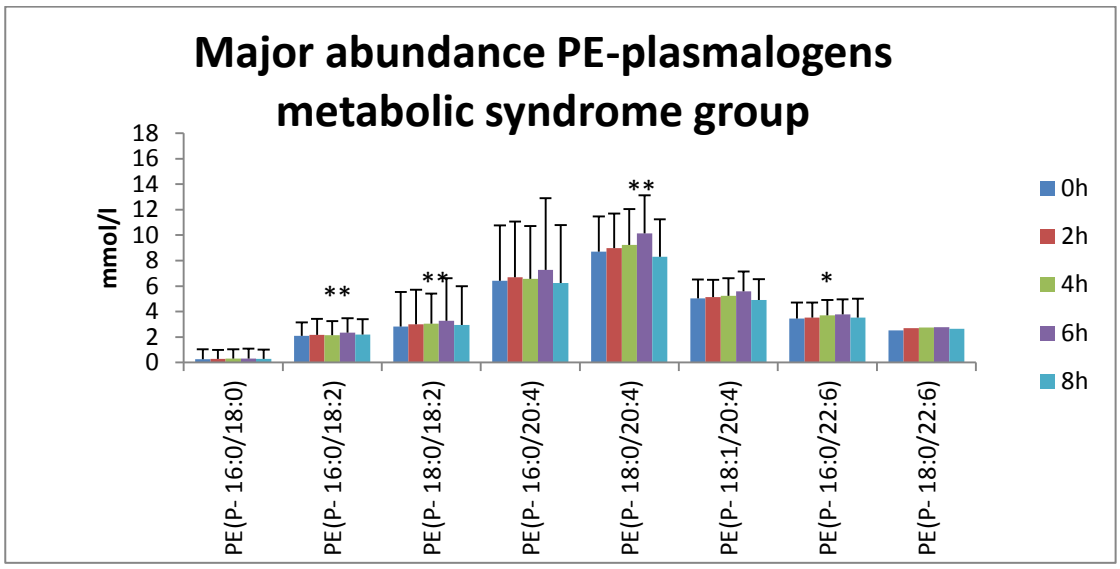


Figure 40: Major-abundance plasmalogens, ± 1 SE

The plasmalogens showed dynamic differences in the three groups. The examined plasmalogen species showed different basal levels in the three groups, and the application of the oral fat tolerance test helped to identify metabolic syndrome subjects. Functionally, the plasmalogens in the plasma serve as lipid supply for the brain, but it has also been proposed that they act as serum antioxidants preventing lipoprotein oxidation [112] and as regulators of membrane fluidity [62]. Predominant species are DHA and AA [112]. Our results also provided that the major species in the plasma compartment of all three groups were AA containing plasmalogens and PE (P-16:0/22:6), PE (P-18:0/22:6) followed by PE (P-16:0/18:2) and PE (P-18:0/18:2).

Species	p-value
PE (P-16:0/16:0)	0.081
PE (P-16:0/18:0)	0.143
PE (P-18:1/18:0)	0.011
PE (P-16:0/16:1)	0.126
PE (P-18:0/16:1)	0.232
PE (P-18:1/16:1)	0.071
PE (P-16:0/18:1)	0.159
PE (P-18:1/18:1)	0.008
PE (P-16:0/18:2)	0.07
PE (P-18:0/18:2)	0.008
PE (P-18:1/18:2)	0.037
PE (P- 18:0/18:3)	0.190
PE (P- 18:1/18:3)	0.26
PE (P- 18:0/20:3)	0.428
PE (P-18:0/22:3)	0.021
PE (P- 18:1/22:3)	0.118
PE (P-16:0/20:4)	0.604
PE (P 18:0/20:4)	0.372
PE (P-18:1/20:4)	0.248
PE (P-16:0/22:4)	0.405
PE (P-18:0/22:4)	0.670
PE (P-18:1/22:4)	0.037
PE (P-16:0/20:5)	0.185
PE (P-18:0/20:5)	0.258
PE (P- 18:1/20:5)	0.935
PE (P- 16:0/22:5)	0.069
PE (P- 18:0/22:5)	0.017
PE (P 18:1/22:5)	0.205
PE (P- 16:0/22:6)	0.607
PE (P- 18:0/22:6)	0.823
PE (P- 18:1/22:6)	0.384

Table 9: Differences between the groups: Plasmalogen species and the p-value of the Kruskal-Wallis test.

Of the plasmalogen species with a saturated or monounsaturated acyl residue in sn-2 position, only the species PE (P-18:1/18:0) and PE (P-18:1/18:1) showed a basal difference between the groups. In the group of the polyunsaturated with 2 and 3 double bonds, the plasmalogens PE (P- 18:0/18:2) and PE (P-18:1/18:2) as well as PE (P-18:0/22:3) showed significant differences between the groups. From the plasmalogens with an acyl residue in sn -2 position with a desaturation degree of 4, only PE (P-18:1/22:4) showed a significant difference between the groups. Looking at the plasmalogens with 5 double bonds, the table shows that there was only a significant difference in the PE (P-18:0/22:5). In the comparison of the plasmalogens in the three groups at time zero, none of the plasmalogen species containing an acyl residue with 6 double bonds in sn2 position showed any difference in concentration.

Plasmalogen	control	risk	metabolic syndrome
PE(P-16:0/16:0)	0.8	0.35	0.008 **
PE(P-18:0/16:1)	0.88	0.2	0.07
PE(P-18:1/16:1)	0.3	0.39	0.02 *
PE(P-18:0/18:1)	0.7	0.008 **	0.006 **
PE(P-18:1/18:1)	0.16	0.3	0.012 *
PE(P-16:0/18:2)	0.3	0.38	0.009 **
PE(P-18:0/18:2)	0.6	0.07	0.003 **
PE(P-18:1/18:2)	0.2	0.2	0.3
PE(P-18:1/20:3)	0.69	0.26	0.007 **
PE(P-18:0/20:4)	0.06	0.006 **	0.001 **
PE(P-18:0/22:4)	0.02 *	0.043 *	0.005 **
PE(P-18:1/22:3)	0.32	0.1	0.03 **
PE(P-16:0/22:5)	0.127	0.042 **	0.05 *
PE(P-18:1/22:5)	0.6	0.4	0.02*
PE(P-16:0/22:6)	0.2	0.3	0.02 *

Table 10: Dynamic over time: Plasmalogens with p-value of Friedman test.

Observation of the development over time revealed that most of the plasmalogen species showed a peak at 6 hours in the metabolic syndrome group. In the control group, most species did not show a response to the fat load, and those which did reached their peak levels earlier than metabolic syndrome subjects. PE (P-18:1/18:1) showed an increase in comparison to time zero in the metabolic syndrome group which did not occur in the control group (Friedman, p=0.16 control, p=0.3 risk; p=0.012, metabolic syndrome). Since there was no recognizable stringency when comparing the dynamics of the plasmalogens with the same fatty acid in sn-2 position, examining the most common plasmalogens individually, would provide more information. In biosynthetic pathways, plasmalogens serve as a reservoir for PUFAs, as they preferentially contain arachidonic acid, DPA and DHA in sn-2 position [62].

Reservoir for PUFA: DHA-containing plasmalogens

With age, the peroxisome function decreases, which leads to a decrease in plasmalogen and DHA synthesis [62]. The half-life of plasmalogens is about 3 hours [62]. A look at the DHA-containing plasmalogens (16:0/ 22:6), for example showed a significant reaction to the OFTT in the metabolic syndrome group. The peak is again 6 hours after ingestion.

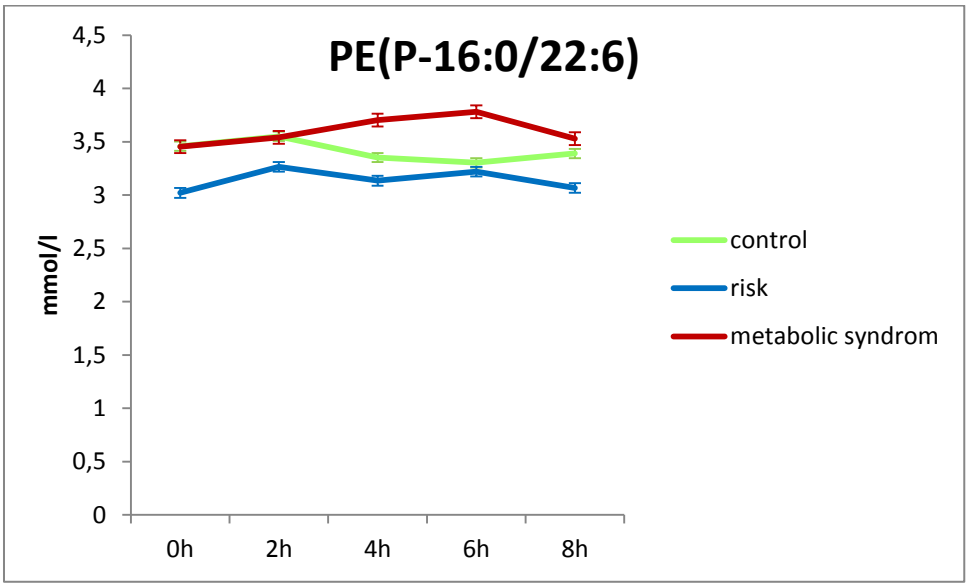


Figure 41: Plasmalogen (16:0/DHA). Kruskal-Wallis test: p=0.6; Friedman, p=0.2, control; p=0.304, risk; p=0.02 metabolic syndrome. The change over time was significantly different in the metabolic syndrome group. Data is represented as means ±1 SE.

Arachidonic acid, linolenic and stearic acid containing plasmalogens

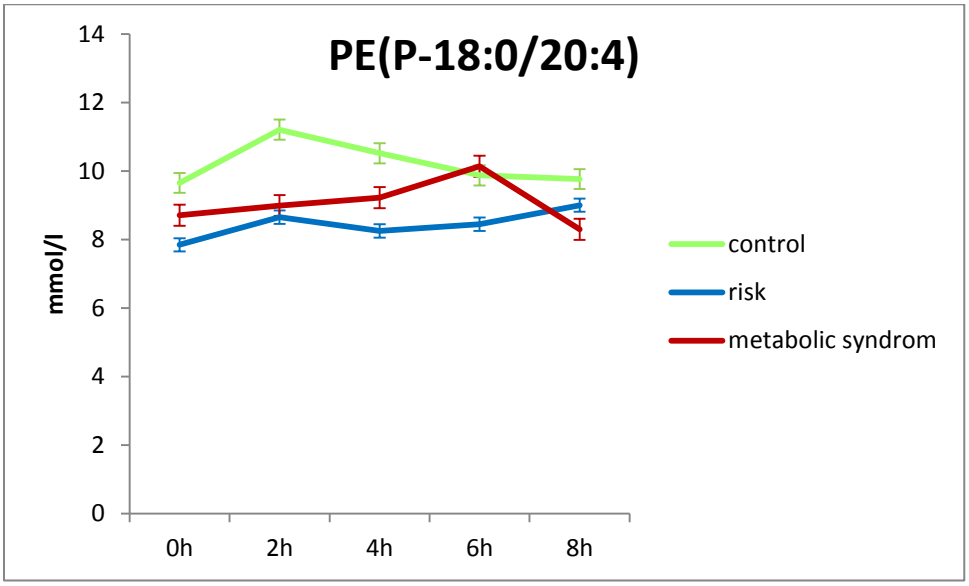


Figure 42: PE (P-18:0/20:4), Kruskal-Wallis test, p= 0.37; Friedman test, p= 0.06, control; p=0.006, risk ; p=0.01; metabolic syndrome. The changes over time were significantly different in the risk and the metabolic syndrome group. Data is represented as means ±1 SE.

The basal differences in AA containing plasmalogens were not significant, but all species with 20:4 at sn-2 position showed the same trend. Over time, the metabolic syndrome and the risk groups showed a significant increase in their levels. Plasmalogens with linoleic acid were down-regulated in metabolic syndrome subjects (S 17) P (PE-18:0/18:2) and P (PE-18:1/18:2) initially showed significantly reduced levels in the metabolic syndrome subjects (Kruskal-Wallis, p= 0.008 and p= 0.037). All of them showed a peak in the metabolic syndrome group 6 hours after ingestion of the drink. The increase was significant in the plasmalogens with acyl residue 16:0 (Friedman, p=0.3, control; p=0.38, risk; p=0.009, metabolic syndrome) and 18:0 (Friedman p=0.6, control; p=0.07, risk; p=0.003) in sn-1 position. The plasmalogen PE (P-16:0/18:0) was analyzed in more detail (supplemental, S 18), because stearic acid has been reported to be elevated in oxidative stress [113]. The plasmalogen 16:0/18:0 concentration showed a significant difference between the control and the risk group (Mann-Whitney-U p= 0.048). Over time, a significant change was observed in the metabolic syndrome group (p=0.001, n=30), while the other groups' levels did not change (p=0.97, control, n=12, control and p=0.93, risk, n=18). Thus, only the metabolic syndrome subjects showed an increase for 6 hours and a decrease after 6 hours.

Correlation of the plasmalogens with the fatty acids

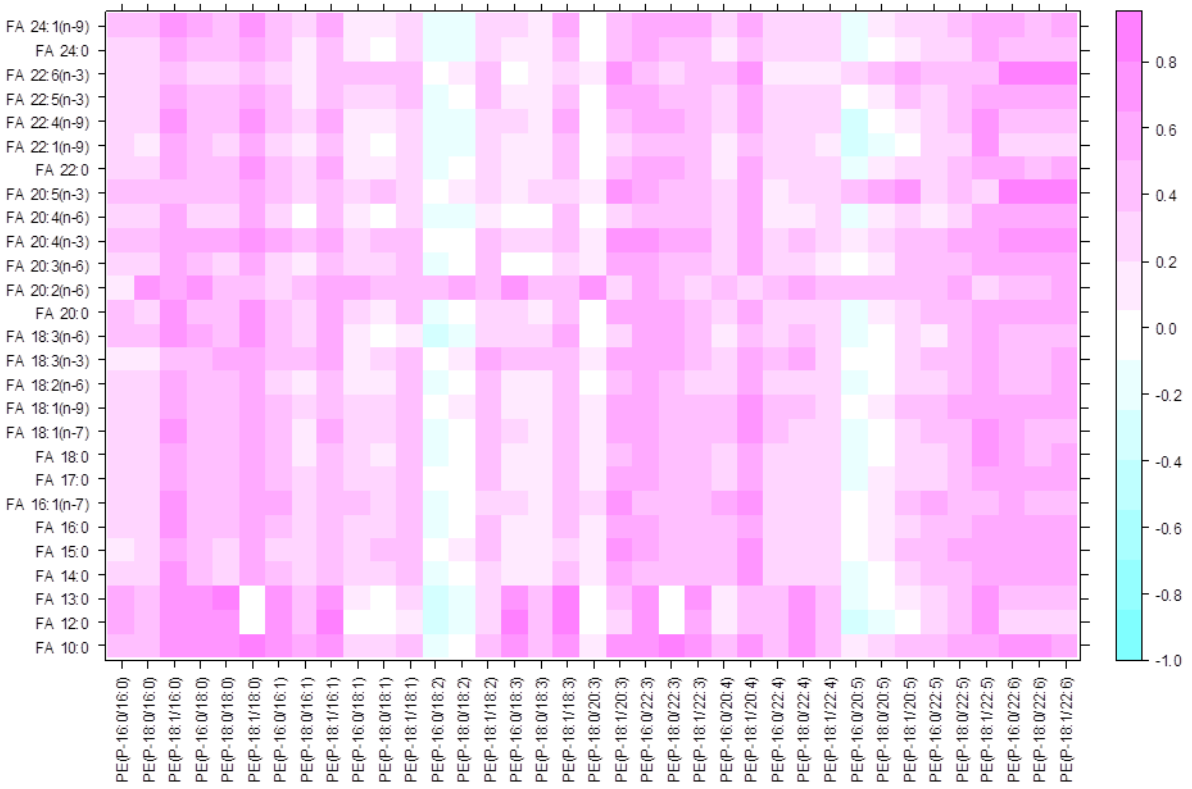


Figure 43: Correlation between plasmalogen species and free fatty acids in the control group. Positive correlation is shown in red, negative correlation in blue. Significance is assumed at correlation coefficients r>0.6.

When correlating the fatty acids with the plasmalogens in the control group (Figure 43) the baseline levels of the plasmalogens with a saturated acyl residue in sn-2 position showed a positive correlation between the short saturated fatty acids FA 10:0 and FA 12:0 and PE (P-18:1/16:0) as well as with the plasmalogens PE (P-16:0/18:0) and PE (P-18:0/18:0). Moreover, a strong positive correlation was observed between the fatty acids 14:0, 16:0, 18:0, 18:1 (n-6), 18:1 (n-7), 18:2 (n-6), 20:0, 22:4 (n-9), 24:0, 24:1 (n-9), 22:5 (n-3) and the plasmalogens PE (P-18:1/16:0) and PE (P-18:1/18:0). Fatty acid 20:2 (n-6) positively correlated with PE (P-18:0/16:0) and PE (P-16:0/18:0). Concerning the plasmalogens with monounsaturated fatty acids in sn-2 position in the control group, the fatty acids 10:0 and 12:0 positively correlated with the plasmalogen species PE(P-16:0/16:1) and (18:1/16:1) in the control group. FA 18:3 (n-6) positively correlated with PE (P-18:1/16:1). FA 20:2 (n-6) positively correlated with PE (P-16:0/18:1). The control group showed a positive correlation between FA 10:0 and FA 12:0 and the plasmalogen species PE (P-16:0/18:3), PE (P-18:1/18:3), PE (P-16:0/22:3), and PE (P-18:1/22:3). The fatty acid 10:0 also correlated positively with PE (P-18:1/20:3). The control group showed a positive correlation between FA 16:1 and PE (P-18:1/20:3). The fatty acid 20:2 (n-6) positively correlated with PE (P-16:0/18:3). The fatty acids 22:0 and 20:4 (n-3) correlated positively with PE (P-16:0/22:3) and FA 20:4 (n-3) correlated positively with PE (P-18:1/22:3) and PE (16:0/22:3). Fatty acid 20:5 (n-3) correlated positively with PE (P-18:1/20:3). The fatty acid 24:1 positively correlated with PE (P-16:0/22:3). The fatty acid 22:6 (n-3) correlated positively with PE (P-18:1/22:3). Concerning the plasmalogens with four double-bonds, in the control group arachidonic acid (20:4 n-6) positively correlated with all the plasmalogens carrying 20:4 in sn-2 position and with PE (P-18:0/22:4) and PE (P-18:1/22:4). The plasmalogen PE (P-18:1/20:4) positively correlated with almost all the fatty acids: FA 10:0, FA 14:0, FA 15:0, FA 16:0, FA 16:1, FA 17:0, FA 18:1 (n-6), FA 18:1 (n-7), FA 20:3 (n-6), FA 20:4 (n-3), FA 20:5 (n-3), FA 22:6 (n-3), DHA (22:6 n-3) correlated positively with the plasmalogen PE (P-18:0/20:4). The medium-chain fatty acids positively correlated with PE (P-18:1/22:4). In the control group, the plasmalogens carrying 5 and 6 double bonds showed that fatty acids 10:0 and 15:0 correlated with plasmalogens carrying 22:6 in sn-2 position. Fatty acid 16:0 positively correlated with the plasmalogen (18:1/22:5). Fatty acids with 17, 18, 22 and 24 C atoms as well as fatty acid 20:0 and 20:3 (n-6) positively correlated with PE (P-18:1/22:5). The fatty acids 20:5 (n-3), 22:5 (n-3), 22:6 (n-3) positively correlated with all of the plasmalogens carrying 22:6 as acyl residue in sn-2 position.

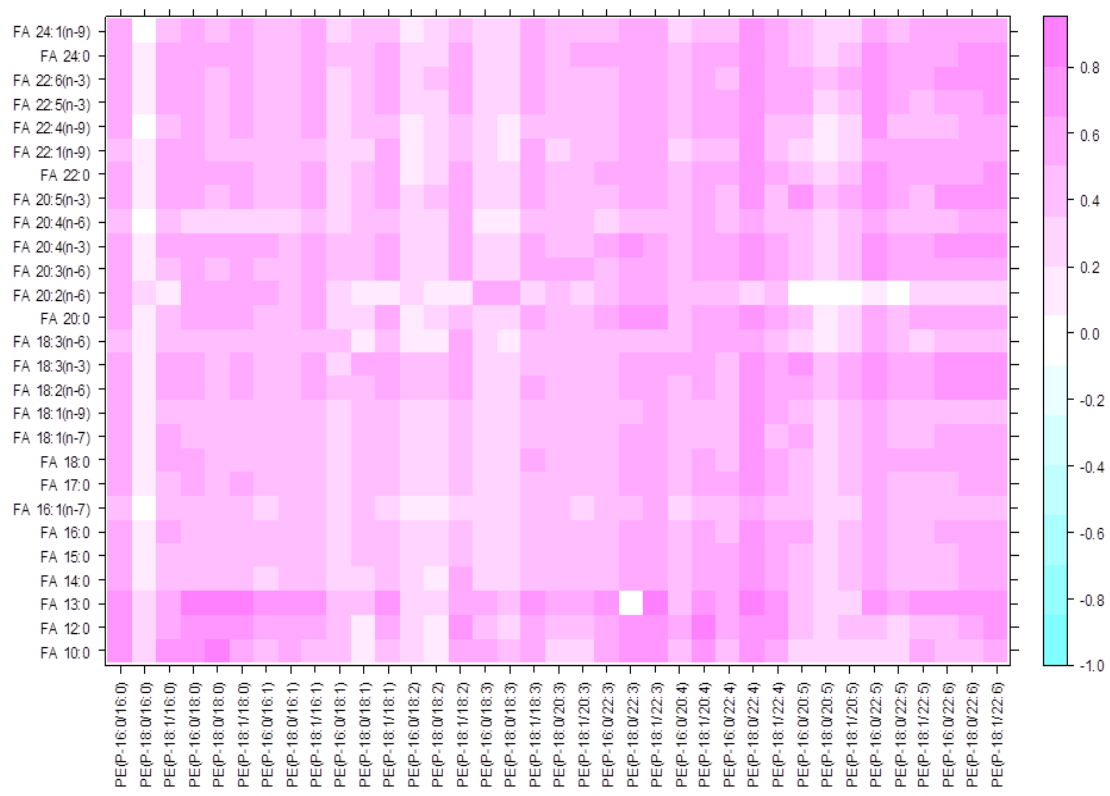


Figure 44: Correlation between plasmalogen species and free fatty acids in the risk group. Positive correlation is shown in red, negative correlation in blue. Significance is assumed at correlation coefficients r>0.6.

In the risk group (Figure 44), the medium-chain fatty acids correlated positively with all plasmalogen species carrying 18:0 in sn-2 position and with PE (P-16:0/16:0). Fatty acid 20:4 (n-3) correlated positively with certain plasmalogens carrying 18:0 and 16:0. Fatty acid 22:0 also correlated positively correlated with PE (P-16:0/18:0). Fatty acid 20:4 (n-6) positively correlated with PE (P-18:0/18:1), but FA 20:4 (n-3) with PE (P-18:1/16:1) and PE (P-18:1/18:1). The risk group showed a positive correlation between FA 10, FA 12 and PE (P-18:0/22:3) and PE (P-18:1/22:3). FA 12:0 positively correlated with the plasmalogen PE (P-18:1/18:3).The fatty acids 20:0 and 20:2 (n-6) positively correlated with plasmalogens PE (P-18:0/22:3) and PE (P-18:1/22:3).The fatty acid 20:2 (n-6) correlated positively with PE (P-18:0/22:3). The risk group showed a positive correlation between FA 20:4 (n-6) and PE (P-16:0/20:3) as well as 20:4 (n-3) and PE (P-18:1/18:3), PE (P-18:0/22:3) and PE (P-18:0/22:3). FA 22:0 also correlated with the latter two plasmalogens. The fatty acid 24:0 and 24:1 correlated with PE (P-18:1/22:3) and fatty acid 24:1 with PE (P-18:0/22:3) as well. The risk group showed a positive correlation between the plasmalogen PE (P-18:1/22:4) and almost all of the fatty acids. PE (P-18:1/22:4) positively correlated with the medium-chain fatty acids. PE (P-18:0/20:4) correlated positively with the medium-chain and saturated long-chain fatty acids, moreover with linolenic, linoleic 20:3 (n-6), 20:4 (n-3), and 20:4 (n-6). The fatty acid

20:4 (n-6) correlated with almost all of the plasmalogens and with all carrying 20:4 in sn-2 position. In the risk group, 12:0 positively correlated with two plasmalogens carrying 22:6 in sn-2 position. Fatty acids 17:0, 18:0, 18:1 (n-7), 18:3 (n-3), 20:3 (n-6), 20:4 (n-6), 22:0, 20:4 (n-3), 22:1 (n-9), 20:5 (n-3), 22:4 (n-3), 22:1 (n-9), 22:4 (n-9), 24:0 and 24:1 (n-9), as well as all the species with 22 C atoms, correlated positively with PE (P-16:0/22:5). The plasmalogens PE (P-18:0/22:6) and PE (P-18:1/22:6) correlated positively with almost all of the fatty acids with the exception of palmitoleic acid, palmitic acid, FA 17:0, the FA 18:1 species, γ-linolenic acid and 22:2 (n-6). In the metabolic syndrome group, we found the most correlations. Since nearly all of the plasmalogens and fatty acids correlated positively in this group, only the strongest correlations which are most relevant to the discussion will be described. The correlations are shown in Figure 45. In the metabolic syndrome group, saturated fatty acids and the plasmalogens containing a saturated fatty acid in sn-2 position showed significant correlation coefficients for the correlations of the fatty acids 10:0, 12:0, 14:0 and all of the plasmalogen species. The fatty acid 20:3 (n-6) positively correlated with PE (P-18:0/16:0), FA 24:1 (n-9) positively correlated with PE (P-18:1/16:0).

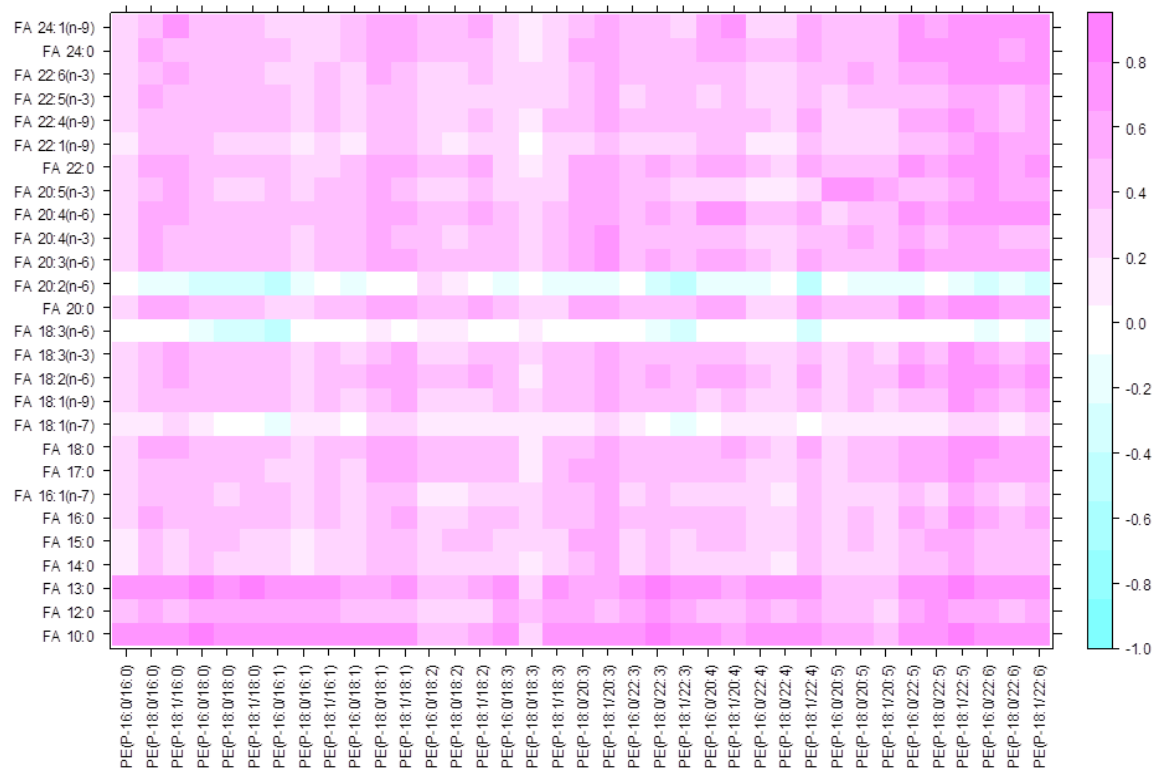


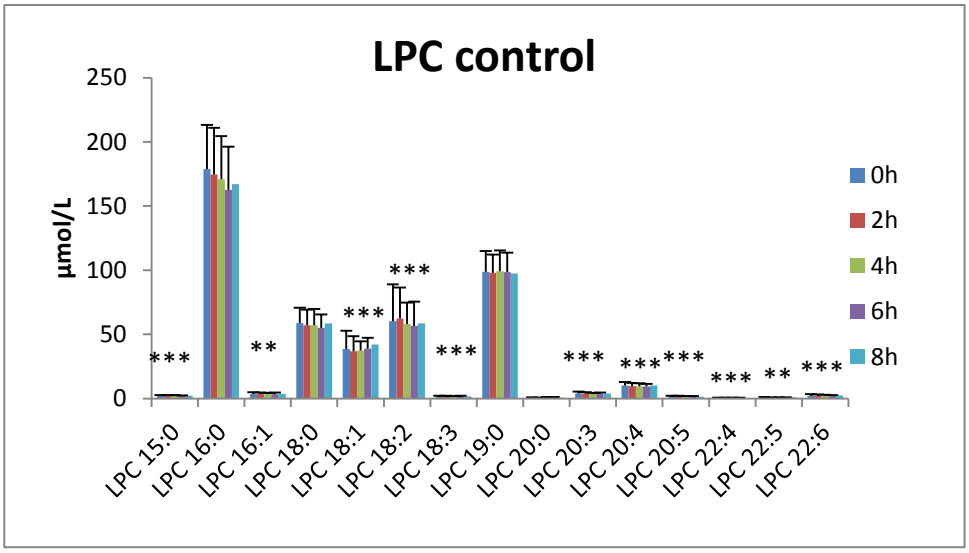
Figure 45: Correlation between plasmalogen species and free fatty acids in the metabolic syndrome group. Positive correlation is shown in red, negative correlation in blue. Significance is assumed at correlation coefficients r>0.6.

The fatty acids 10:0 correlated with all plasmalogen species, fatty acid 13:0 with all the plasmalogens carrying 16:1 as an acyl residue in sn-2 position and with the plasmalogen PE (P-18:1/18:0). The fatty acid 12:0 positively correlated with the PE (P-18:1/16:1), fatty acid 24:1 (n-9) positively correlated with PE (P-18:0/18:1) and PE (P-18:1/18:1). Concerning the plasmalogens with three double bonds in the metabolic syndrome group, a positive correlation was found between the fatty acid 10:0 and the plasmalogens PE (P-16:0/18:3), PE (P-18:1/18:3), PE (P-18:0/20:3), PE (P-18:1/20:3), PE(P-16:0/22:3), PE(P-18:0/22:3), PE (P-18:1/22:3); fatty acid 12:0 and PE (P-18:0/22:3); fatty acid 18:0 and PE(P-18:1/20:3), FA 18:1 (n-9) and PE (P-18:1/20:3). Fatty acid 20:4 (n-3) positively correlated with PE (P-18:0/20:3). PE (18:1/20:3) positively correlated with most of the fatty acids. Fatty acid 13:0 positively correlated with the plasmalogens PE (P-16:0/18:3), PE (P-18:1/18:3), PE(P-18:1/20:3), PE (P-16:0/22:3), PE (P-18:0/22:3), PE (P-18:1/22:3), fatty acid 17:0 with the plasmalogen PE(P-18:1/20:3). All of the plasmalogens carrying 22:6 in sn-2 position correlated with the fatty acids 20:5 (n-3), 24:0, 24:1 (n-9), 22:6 (n-3), 22:0 20:0, 18:2 (n-6). PE (P-18:1/22:5) correlated positively with all of them as well, and in addition with FA 22:5 (n-3). FA 20:5 (n-3) correlated positively with all plasmalogens carrying 20:5 in sn-2 position and all carrying 22:6. Linoleic 18:2 (n-6) and α-linolenic 18:3 (n-3) positively correlated with the plasmalogens carrying DHA in sn-2 position in the risk and metabolic syndrome groups, contrary to γ-linolenic acid which did not positively correlate with any of the plasmalogen species in these groups, but showed a discrete yet not significant negative correlation value in the metabolic syndrome group when correlated to the named plasmalogen species. Arachidonic acid 20:4 (n-6) correlated positively in the control and risk groups with the named plasmalogen species, e.g. species carrying 22:4 in sn-2 position, whereas in the metabolic syndrome group it only correlated positively with plasmalogens containing 20:4. Linoleic acid (18:2 n-6) positively correlated with PE (P-18:1/20:4) in the risk and metabolic syndrome groups.

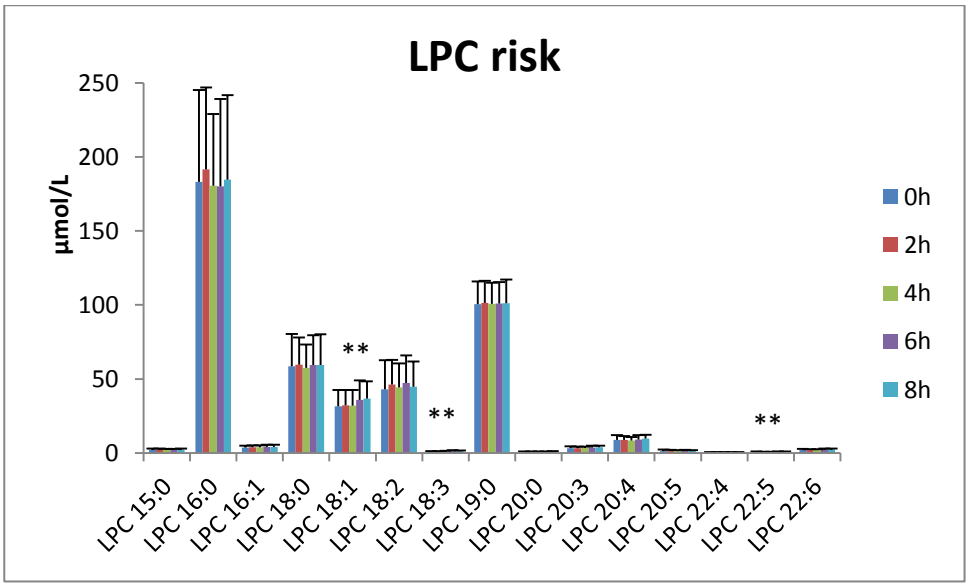
3.3.7 Lysophosphatidylcholine, lysophosphatidic acid

Following these observations, lysophosphatidylcholine (LPC) and lysophosphatidic acid (LPA) were examined. LPC species play an important role as G-protein receptor ligands and are involved in regulating cellular proliferation and tumor cell invasion. They also have atherogenic and proinflammatory properties [65], [114]. Figure 46 provides an overview of the different LPC species and their dynamics over time in the different groups.

A



B



C

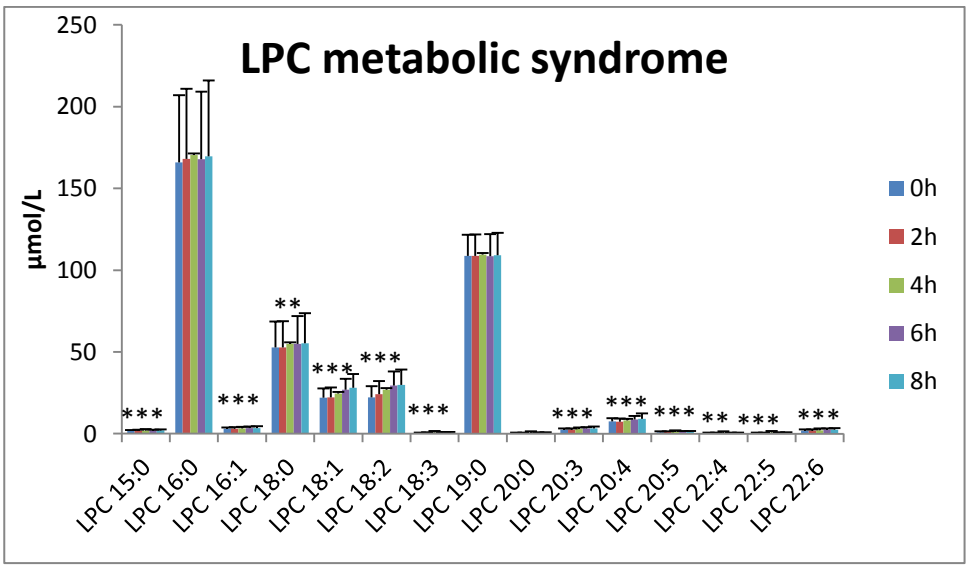


Figure 46: LPC species over time. A) Control group B) risk group C) metabolic syndrome group. Data is represented as means ± 1SD.

As obesity is associated with chronic low-grade inflammation, one might expect LPC levels to be elevated in obesity. Indeed, certain plasma LPC species have been reported to be raised in obesity and type 2 diabetes [65,114]. When comparing the groups, we found significant lower baseline levels of the LPC species 18:1, 18:2, 18:3 in the metabolic syndrome group than in the control and the risk groups (Figure 46). In addition, the levels of the LPC species 20:3, 20:4, 20.5, 22:5 could be observed to be down-regulated in the metabolic syndrome group (Figure 46). P-values are depicted below (Table 11).

LPC	15:0	16:0	16:1	18:1	18:2	18:3	18:0	19:0	20:0	20:3	20:4	20:5	22:4	22:5	22:6
p	0.063	0.429	0.077	0.000	0.000	0.000	0.357	0.013	0.205	0.002	0.009	0.007	0.716	0.000	0.480

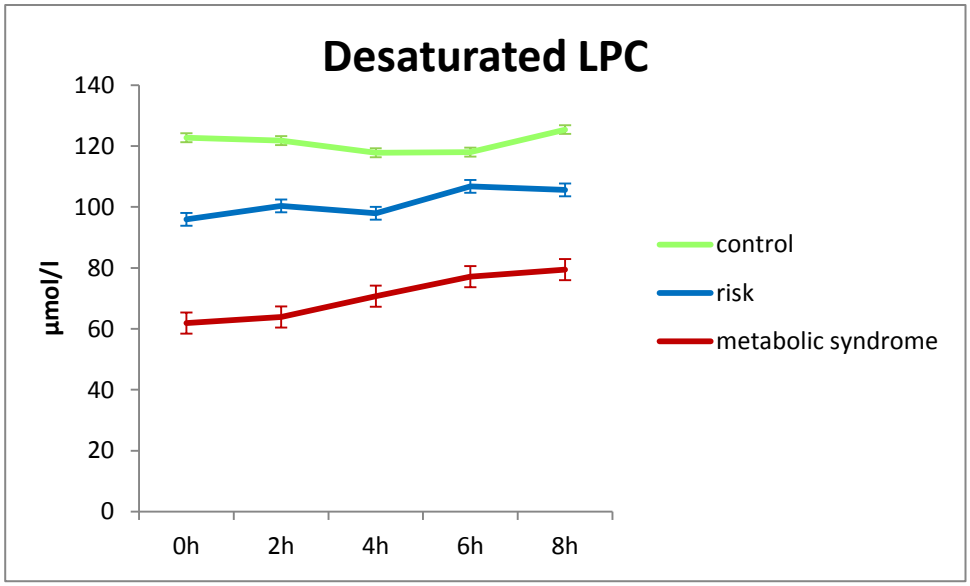
Table 11: LPC species with p-value between the groups of the Kruskal-Wallis test.

The differences in the behavior of the latter – and of other important species – will later be explicated in detail, and a relationship will be established between the PC and those fatty acid species necessary to form the respective phosphatidylcholine (PC) with the LPC species. First, an overview of the behavior of desaturated LPC and total LPA species is shown in Figure 47.

Figure 47 A shows the differences in desaturated lysophosphatidylcholine species between the groups. The metabolic syndrome group showed significantly decreased values compared to the other groups ($p<0.001$). This was tested by Kruskal-Wallis. Over time, the results showed the following p values: $p=0.92$, control, $n=12$; $p=0.13$, risk, $n=18$; $p<0.001$ metabolic syndrome, $n=31$. Thus, only the metabolic syndrome group responded to the drink with increasing values.

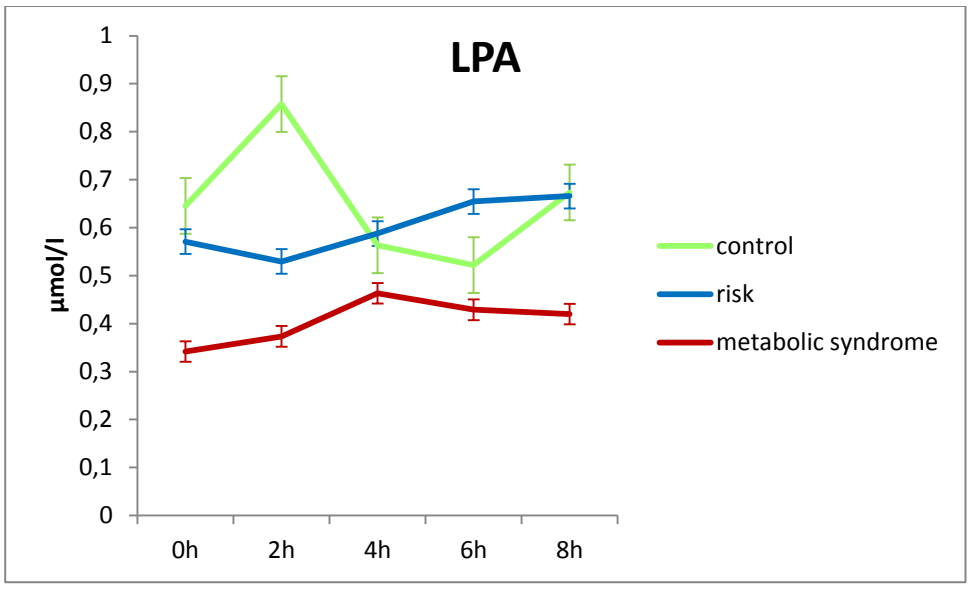
Figure 47 B shows the mean LPA values. There was a significant difference between the groups (Kruskal-Wallis test, $p=0.016$, $n=23$). The metabolic syndrome group had lower basal values than the control and the risk groups. The metabolic syndrome subjects also reacted to the drink (Friedman, $p=0.78$, control, $n=$; $p=0.51$, risk, $n=9$; $p=0.026$, metabolic syndrome, $n=6$). Thus, over time, the only significant increase was after four hours in the metabolic syndrome group.

A



Kruskal-Wallis test n=61, Friedman test n=12 control, n=18 risk, n=31, metabolic syndrome.

B



Kruskal Wallis: n=23, Friedman: n=7 control; n=9 risk; n=6 metabolic syndrome.

Figure 47: Lysophopholipids. A) Comparison of LPC (unsaturated species) distribution between the groups and over time. There was a significant difference between the groups. Over time, the metabolic syndrome group responded to the drink with increasing values. B) Comparison of LPA distribution between the groups and over time. Distribution between the groups was compared using Kruskal-Wallis; changes over time were tested by Friedman. There was a significant difference between the groups. Over time, the metabolic syndrome group responded to the drink with increasing values. Data are represented as means ± 1SE.

Unsaturated lysophosphatidylcholine and lysophosphatidic acid both showed decreased baseline values in the metabolic syndrome group. The oral fat tolerance test provided additional information to identify metabolic syndrome subjects.

LPC	Control	Risk	Metabolic syndrome
LPC 15:00	<0.001	0.11	<0.001
LPC 16:00	0.26	0.057	0.7
LPC 16:01	0.004	0.647	<0.001
LPC 18:00	0.58	0.344	0.004
LPC 18:01	<0.001	0.009	<0.001
LPC 18:02	<0.001	0.247	0.001
LPC 18:03	<0.001	0.002	0.001
LPC 19:00	0.54	0.8	0.80
LPC 20:00	0.8	0.54	0.59
LPC 20:03	<0.001	0.13	<0.001
LPC 20:04	<0.001	0.058	<0.001
LPC 20:05	<0.001	0.571	0.001
LPC 22:04	0.007	0.51	0.009
LPC 22:05	<0.001	0.07	<0.001
LPC 22:06	<0.001	0.11	<0.001

Table 12: p-values calculated using the Friedman test for the LPC species of all three groups.

Looking at LPC 18:0, a saturated LPC species, the change over time as a response to the OFTT only occurred in the metabolic syndrome group (Table 12). Therefore the OFTT could be applied to determine acute changes in metabolism which indicate that the respective subject has the properties of subjects belonging to the metabolic syndrome group. Thus, by reviewing the results, it may be possible to classify him or her into this group. LPC species 18:0 has already been described in other examinations to be one of the rare LPC species that should be induced in rodents after a long-term high fat diet [115]. In these results, shown in supplemental, (S 19), no significant differences in the baseline level could be observed when comparing the groups (Kruskal-Wallis $p=0.3$), but over time the metabolic syndrome group showed an increase ($p=0.004$). In a next step, the desaturated LPC species with either chronically reduced or acutely changed levels over time in the metabolic syndrome group were depicted and described in detail (S 20, S 21, S 22, S 23, S 24, S 25, S 26). Reduced levels of LPC 18:1 in obese men have already been described [115]. According to those examinations, S 20 showed down-regulated baseline levels in the metabolic syndrome group, while LPC 18:1 levels over time were induced in all three groups as a response to the OFTT. S 21 also shows the decreased baseline level of LPC 18:3 in the metabolic syndrome group ($p<0.001$) and an increase over time in all three groups with the same peak at 6 hours ($p<0.001$ control, $p=0.002$ risk, $p<0.001$). This species will be referred to again below in the

context of changes over time in phosphatidylcholine (PC) concentration and the corresponding fatty acid that builds the PC species together with the LPC. LPC 22:4 (S 22) was increased after the OFTT in the control and the metabolic syndrome groups. This is in agreement with other examinations showing a long-term increase in LPC 22:4 levels in mice after a high-fat diet [115]. In the risk group, we did not find a significant increase, but as a trend was recognizable. Next, polyunsaturated LPC species with 20 C atoms were described (S 23, S 24, S 25). LPC 20:3, 20:4 and 20:5 all showed significantly decreased levels in the metabolic syndrome group, compared to the other groups. Over time, all of them showed a significant change in the control and also in the metabolic syndrome group. The LPC species LPC 22:5 also showed a significantly decreased level in the metabolic syndrome group and a significant change over time in all groups. Before we compared the LPC to the PC levels and to the appropriate fatty acid species that build the PC species with LPC, a closer look was taken at LPA.

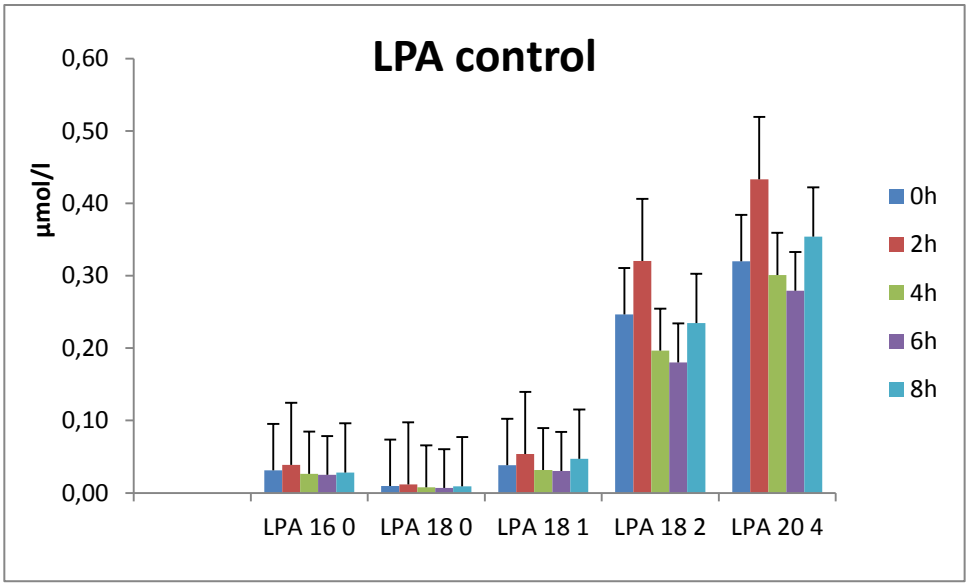
LPA has previously been described as correlating positively with diabetes [116]. LPC can be converted into LPA. Simultaneously to LPA secretion, adipocytes release an LPA-synthesizing activator, which has been characterized as a lysophospholipase D activity and which catalyzes the transformation of lysophosphatidylcholine into LPA [117]. Figure 48 provides an overview of the different LPA species and their dynamics over time in the different groups.

Kruskal-Wallis, LPA 16:0 (palmitate), p=0.34; LPA 18:0 (stearate), p=0.265; LPA 18:1 (oleate), p=0.009, LPA 18: 2 (linoleate), p=0.005, LPA 20:4 (arachidate), p=0.305.

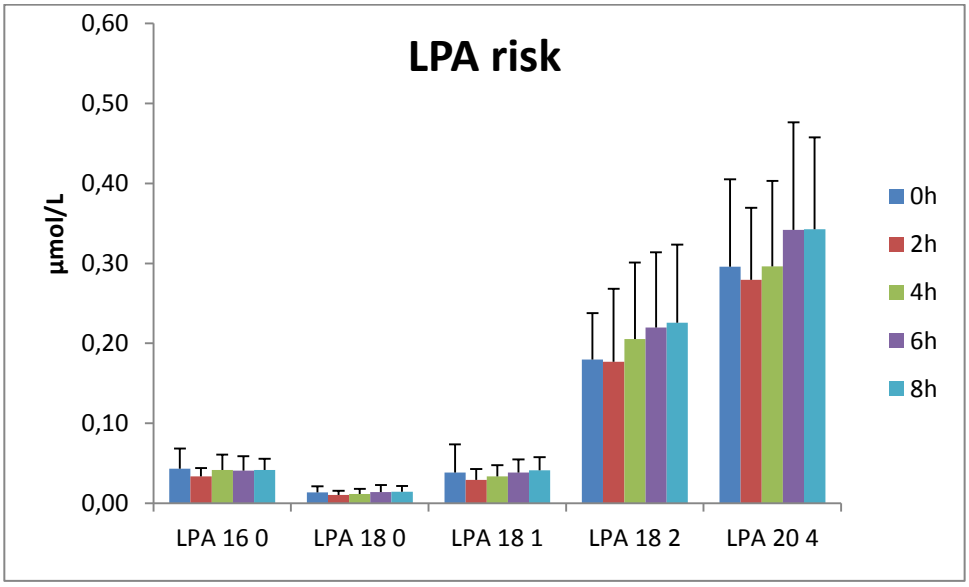
LPA species	Control	Risk	Metabolic syndrome
LPA 16:0	0.63	0.31	0.5
LPA 18:0	0.65	0.17	0.32
LPA 18:1	0.63	0.23	0.029
LPA 18:2	0.94	0.32	0.017
LPA 20:4	0.9	0.31	0.7

Table 13: p-values calculated using the Friedman test for LPA species of all three groups

A



B



C

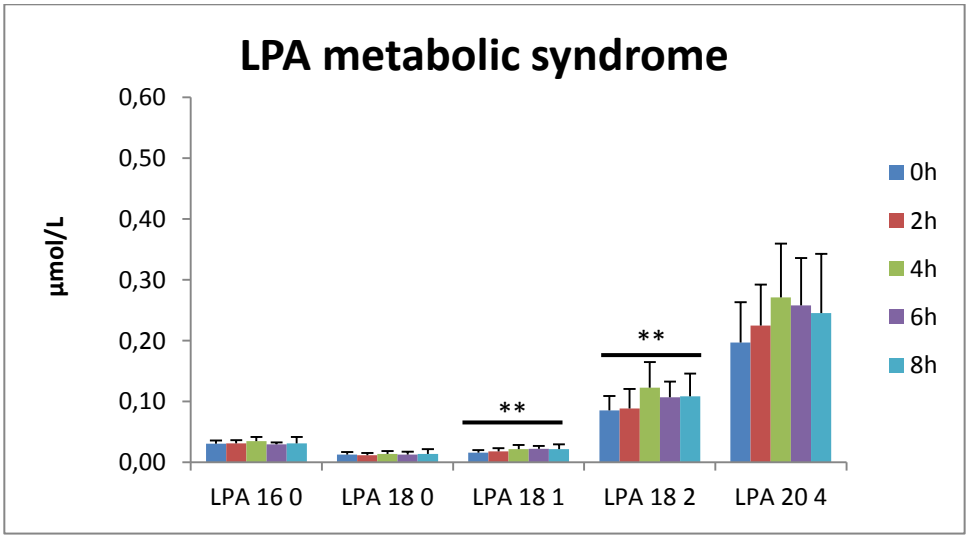


Figure 48: LPA species over time. A) Control group B) risk group C) metabolic syndrome group. Data i s represented as means ± 1SD.

The LPA 20:4 species is a potent peroxisome proliferator-activated receptor gamma (PPARγ) ligand, which is a key regulator of atherogenesis [118]. However, our data record (Figure 48) showed that there is neither a significant difference in the LPA 20:4 concentrations between the groups (Kruskal-Wallis, p=0.3), nor over time (Friedman, p=0.9, control, p=0.7 metabolic syndrome). The LPA species carrying oleate and linoleate therefore showed a significant difference between the groups with down-regulated levels in the metabolic syndrome group. The total LPA concentration is better suited for classifying the groups than the different LPA species (Figure 47 B). The changes in LPC and LPA levels over time relative to baseline levels were depicted (Table 14) and compared in terms of direction of change (red: up, blue: down), since LPA can be produced out of the other lysophospholipids (e.g. LPC) via autotaxin (ATX) activity [119].

A	**LPA 18:0**	**FA 18:0**	LPC 18:0
	LPA 18:1	FA 18:1	LPC 18:1
	LPA 18:2	FA 18:2	LPC 18:2
	LPA 20:4	FA 20:4	LPC 20:4
B	**LPA 18:0**	**FA 18:0**	LPC 18:0
	LPA 18:1	FA 18:1	LPC 18:1
	LPA 18:2	FA 18:2	LPC 18:2
	LPA 20:4	FA 20:4	LPC 20:4
C	**LPA 18:0**	**FA 18:0**	LPC 18:0
	LPA 18:1	FA 18:1	LPC 18:1
	LPA 18:2	FA 18:2	LPC 18:2
	LPA 20:4	FA 20:4	LPC 20:4
D	LPA 18:0	**FA 18:0**	LPC 18:0
	LPA 18:1	FA 18:1	LPC 18:1
	LPA 18:2	FA 18:2	LPC 18:2
	LPA 20:4	FA 20:4	LPC 20:4

Table 14: Control group, LPA (left), fatty acid (middle), LPC (right). Red indicates an increase, blue a decrease, black no change compared to the baseline level. A change compared to time zero is considered if it is ≥1.2, A) 2h, B) 4h, C) 6h, D) 8h.

As a response to the OFTT, LPA 18:0 (Figure 48 and Table 14) and fatty acid 18:0 (S 3 and Table 14) levels both increased two hours after the application of the OFTT. Subsequently, LPA 18:0 decreased while fatty acid 18:0 increased further and the LPC level did not change. This could indicate a conversion of this LPA species either into another one or into

phospholipid. Even though the change in the control group for the FA 18:0 was not described as significant above, in this section, an increase of the fatty acid 18:0 at 2 hours was found, since change was defined as 1.2 times the basal value.

A	**LPA 18:0**	**FA 18:0**	LPC 18:0
	LPA 18:1	FA 18:1	LPC 18:1
	LPA 18:2	FA 18:2	LPC 18:2
	LPA 20:4	FA 20:4	LPC 20:4
B	LPA 18:0	**FA 18:0***	LPC 18:0
	LPA 18:1	FA 18:1	LPC 18:1
	LPA 18:2	FA 18:2	LPC 18:2
	LPA 20:4	FA 20:4	LPC 20:4
C	LPA 18:0	**FA 18:0***	LPC 18:0
	LPA 18:1	FA 18:1	LPC 18:1
	LPA 18:2	FA 18:2	LPC 18:2
	LPA 20:4	FA 20:4	LPC 20:4
D	LPA 18:0	FA 18:0	LPC 18:0
	LPA 18:1	FA 18:1	LPC 18:1
	LPA 18:2	FA 18:2	LPC 18:2
	LPA 20:4	FA 20:4	LPC 20:4

Table 15: LPA (left), fatty acid (middle), LPC (right). Red indicates an increase, blue a decrease, black no change compared to the baseline level. A change compared to time zero is defined as ≥ 1.2. Risk A) 2h, B) 4h, C) 6h, D) 8h.

For the risk group, this protection mechanism – i.e. the conversion of LPA into other species or classes – seems to occur earlier. LPA 18:0 and 18:1 were already down-regulated at 2 hours while the fatty acid 18:0 was up-regulated.

In the metabolic syndrome group (Table 16) the increase in stearic acid, and probably also the consecutive increase in LPA species containing the desaturation products of this fatty acid (oleic and linoleic acid), seemed to be insufficient to extract stearic acid from the plasma; the respective LPC species also showed increased levels. This could indicate a conversion of the LPA species into the respective LPC species. The conversion of LPA 18:0 into other species did not result in a decrease of LPA 18:0 since the level of stearic acid in the metabolic syndrome group is so high that it could not be sufficiently eliminated. Stearic acid is increased and is included in LPA, LPA 18:0 is then converted into LPA species 18:1 and 18:2; the fatty acid concentration in the plasma remains increased, and neither does the LPA 18:0 concentration in the plasma decrease, which can be interpreted as an insufficient elimination

of the fatty acid; eventually LPC concentrations increase, which are probably conversion products.

A	LPA 18:0	**FA 18:0**	LPC 18:0
	LPA 18:1*	18:1	LPC 18:1
	LPA 18:2	18:2	LPC 18:2
	LPA 20:4	20:4	LPC 20:4
B	LPA 18:0	**18:0****	LPC 18:0
	LPA 18:1*	18:1	**LPC 18:1*****
	LPA 18:2	18:2	**LPC 18:2*****
	LPA 20:4	20:4	LPC 20:4**
C	LPA 18:0	**18:0***	LPC 18:0
	LPA 18:1*	18:1	**LPC 18:1*****
	LPA 18:2	18:2	**LPC 18:2*****
	LPA 20:4	20:4	LPC 20:4**
D	LPA 18:0	**18:0**	LPC 18:0
	LPA 18:1	18:1	**LPC 18:1*****
	LPA 18:2	18:2	**LPC 18:2*****
	LPA 20:4	20:4	**LPC 20:4***

Table 16: Metabolic syndrome, LPA (left), fatty acid (middle), LPC (right). Red indicates an increase, blue a decrease, black no change compared to the baseline level. A change compared to time zero is considered if is ≥1.2. A) 2h, B) 4h, C) 6h, D) 8h.

Another way of LPA production is by conversion of phosphatidic acid (PA) [119]. The precursor phospholipids (PC, PS, or PE) can be converted into their corresponding lysophospholipids such as lysophosphatidylcholine (LPC), lysophosphatidylserine (LPS), or lysophosphatidylethanolamine (LPE) [120]. Next, correlation of PC to LPC and the corresponding FA were looked at.

Correlations of PC to LPC and fatty acids

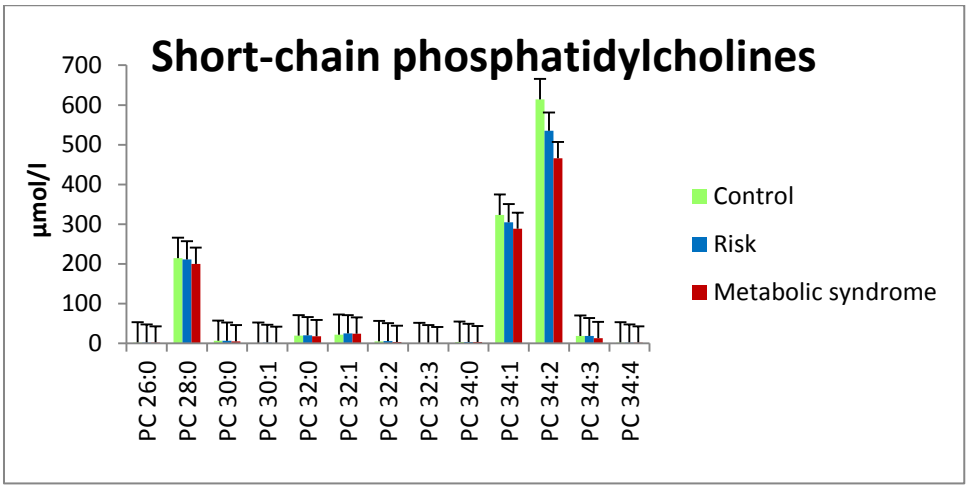


Figure 49: Phosphatidylcholines with <34 C atoms at baseline level

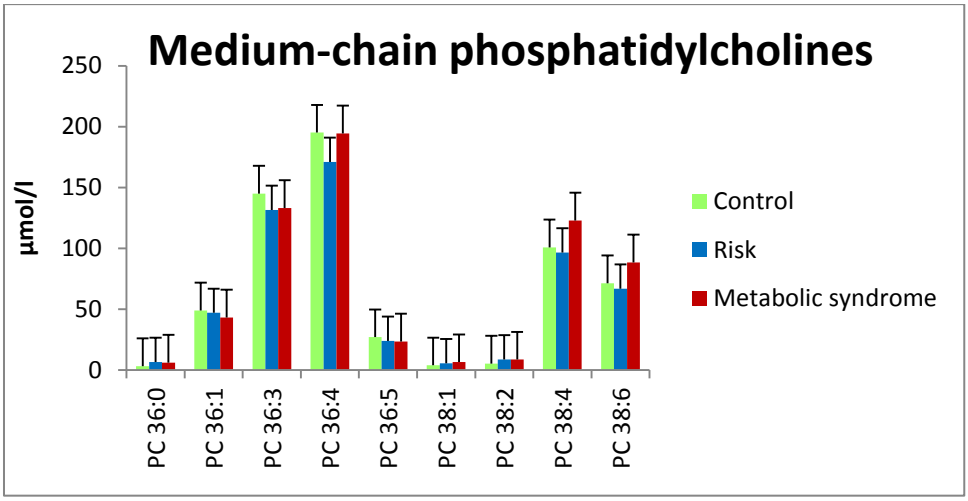


Figure 50: Phosphatidylcholines with 36-38 C atoms at baseline level

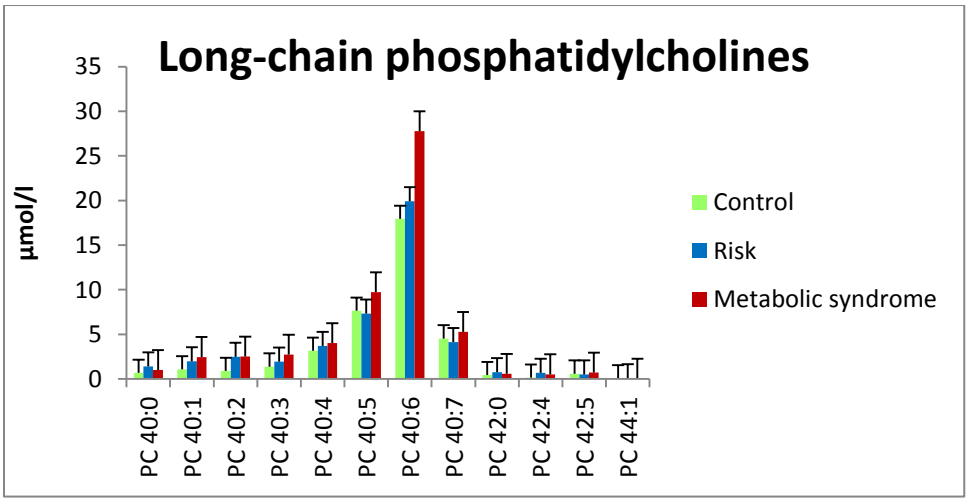


Figure 51: Phosphatidylcholines with >40 C atoms at baseline level

	LPC 20:0	LPC 19:0	LPC 18:0	LPC 18:1	LPC 16:1	LPC 18:2	LPC 20:3	LPC 18:3	LPC 22:4	LPC 20:4	LPC 22:5	LPC 20:5	LPC 22:6
PC 40:7				22:6		22:5	20:4	22:4	18:3	**20:3**	18:2	20:2	18:1
PC 38:6				20:5	22:5	20:4	18:3	**20:3**		18:2	16:1	18:1	**16:0**
PC 40:6			22:6	22:5		22:4	**20:3**		18:2	20:2	18:1		**18:0**
PC 36:5					20:4	18:3		18:2	**14:1**	16:1	**14:0****	**16:0**	
PC 40:5	20:5		22:5	22:4			20:2		18:1		**18:0**	20:0	
PC 42:5	22:5									22:1	20:0	22:0	
PC 34:4					18:3		**14:1**	16:1	**12:0*****	**14:0****			
PC 36:4				18:3	**20:3**	18:2	16:1	18:1	**14:0****	**16:0**			
PC 38:4			20:4	**20:3**		20:2	18:1		**16:0**	**18:0**			
PC 40:4	20:4		22:4					22:1	**18:0**	20:0			
PC 42:4	22:4						22:1	24:1	20:0	22:0			
PC 32:3						**14:1**	**12:0*****	**14:0****					
PC 34:3					18:2	16:1	**14:0****	**16:0**					
PC 36:3			18:3	18:2	20:2	18:1	**16:0**	**18:0**					
PC 40:3	**20:3**					22:1	20:0	22:0					
PC 32:2				**14:1**	16:1	**14:0****							
PC 34:2				16:1	18:1	**16:0**							
PC 38:2	18:2		20:2		22:1	20:0							
PC 40:2	20:2			22:1	24:1	22:0							
PC 30:1				**12:0*****	**14:0****								
PC 32:1			**14:1**	**14:0****	**16:0**								
PC 34:1	**14:1**		16:1	**16:0**	**18:0**								
PC 36:1	16:1		18:1	**18:0**	20:0								
PC 38:1	18:1			20:0	22:0								
PC 40:1			22:1	22:0	24:0								
PC 44:1	24:1												
PC 26:0													
PC 28:0			**10:0*****										
PC 30:0	**10:0*****		**12:0*****										
PC 32:0	**12:0*****	13:0	**14:0****										
PC 34:0	**14:0****	**15:0***	**16:0**										
PC 36:0	**16:0**	**17:0**	**18:0**										
PC 40:0	20:0		22:0										
PC 42:0	22:0		24:0										

Table 17: Control (2h): PC, LPC and FA level at 2 hours were compared to baseline value by calculating the difference between the mean at two hours and at starting point. Increased levels compared to baseline are highlighted in bold (red), decreased levels compared to baseline are highlighted in light grey (blue), and those with no significant changes black. In the middle boxes, those fatty acid species are shown from which the respective PC species could potentially be built with the corresponding LPC species (at the top). PC species are shown on the left. A significant decrease is defined as >1.2. Additionally, the significance we calculated with is shown in stars: : *<0.05, **<0:01, ***<0.001, (scheme based on description by Thomas Kopf).

Table 17 showed a downregulation of the PC species 32:3, 42: 5, 38:1 and 44:1 as a response to the OFTT in the control group, whereas 42:4 and 42:0 were increased by the OFTT. Simultaneously, the fatty acid species FA 10, FA 12, FA 14, FA 15, FA 16, FA 17, FA 18, FA 20:3 and FA 14:1 increased after the fat load (an increase is defined here as difference to the baseline level of >1.2, even if this may not be significant in a t-test or Friedman test). The decrease of PC 32:3 and increase of FA 14:0, 14:1 and FA 12:0 could be interrelated. The cleavage either resulted in increased LPC 20:3, 18:2 or 18:2. This could not be proved by the results. Whereas the oral fat response resulted in reduced PC 32:3 levels after the OFTT in the control group despite an increase in the medium-chain fatty acid concentration, no change in level could be observed in the risk group for this PC after 2 hours; after 4 hours, the level was even increased. This could be interpreted as a protective mechanism, a conversion of 32:3 into another lipid to fill up the PC reservoir with the medium-chain fatty acids again in the control group. In the metabolic syndrome group, PC 32:3 is instantly increased, which suggests a breakdown of the suggested protective mechanism. PC 32:2, a protective species associated with reduced mortality [121], was increased as a response to the OFTT in the control group after 4 hours, (supplement). The palmitoleic acid 16:1 (n-7) level was also increased. It is possible that palmitoleic acid is inserted in 32:2 and by this way withdrawn from plasma. The

development over time is graphically represented in the supplement. After 6 hours (S 28), 32:1 – another PC associated with a reduced mortality compared to PC 32:0 [121] – was increased. In the risk and metabolic syndrome groups, PC 32:2 also showed increased levels as a response to the OFTT, but without an increase of PC 32:1 in the metabolic syndrome group. After four hours (S 27), PC 40:2 increased significantly in the control group whereas the fatty acid 20:2 decreased. This could be interpreted as an integration of this fatty acid into PC 40:2. LPC 20:0 showed a tendency towards decreased levels, which is not significant but visible. In the metabolic syndrome group, PC 40:2 was not up regulated (S 33, S 34, S 35), which might be due to the initially much higher baseline level (Figure 51). After 6 hours, the fatty acid species 22:1, 20:0, 22:0 were increased, LPC 20:0 was induced while PC 42:5 was still decreased. PC 32:1 also showed increased levels as a response to the increase of the fatty acids 14:1, 14:0 and 16:0. After 8 hours (S 29), LPC 18:3 increased, while PC 32:3 decreased and the fatty acid 14:0 increased. This supports the hypothesis that PC is cleaved into the fatty acid and the LPC species eight hours after the OFTT. The fact that the level of PC 42:5 was reduced 8 hours after the fat load indicates that docosapentaenoic acid (22:5) containing PCs released the protective fatty acid.

	LPC 20:0	LPC 19:0	LPC 18:0	LPC 18:1	LPC 16:1	LPC 18:2	LPC 20:3	LPC 18:3	LPC 22:4	LPC 20:4	LPC 22:5	LPC 20:5	LPC 22:6
PC 40:7				22:6		22:5	20:4	22:4	18:3	**20:3**	18:2	**20:2**	18:1
PC 38:6				20:5	22:5	20:4	18:3	**20:3**		18:2	16:1	18:1	**16:0**
PC 40:6			22:6	22:5		22:4	**20:3**		18:2	**20:2**	18:1		**18:0**
PC 36:5					20:4	18:3		18:2	**14:1**	16:1	**14:0****	**16:0**	
PC 40:5	20:5		22:5	22:4			**20:2**		18:1		**18:0**	20:0	
PC 42:5	22:5									22:1	20:0	22:0	
PC 34:4					18:3		**14:1**	16:1	**12:0*****	**14:0****			
PC 36:4				18:3	**20:3**	18:2	16:1	18:1	**14:0****	**16:0**			
PC 38:4			20:4	**20:3**		**20:2**	18:1		**16:0**	**18:0**			
PC 40:4	20:4		22:4					22:1	**18:0**	20:0			
PC 42:4	22:4						22:1	24:1	20:0	22:0			
PC 32:3						**14:1**	**12:0*****	**14:0****					
PC 34:3					18:2	16:1	**14:0****	**16:0**					
PC 36:3			18:3	18:2	**20:2**	18:1	**16:0**	**18:0**					
PC 40:3	**20:3**					22:1	20:0	22:0					
PC 32:2				**14:1**	16:1	**14:0****							
PC 34:2				16:1	18:1	**16:0**							
PC 38:2	18:2		**20:2**		22:1	20:0							
PC 40:2	**20:2**			22:1	24:1	22:0							
PC 30:1				**12:0*****	**14:0****								
PC 32:1			**14:1**	**14:0****	**16:0**								
PC 34:1	**14:1**		16:1	**16:0**	**18:0**								
PC 36:1	16:1		18:1	**18:0**	20:0								
PC 38:1	18:1			20:0	22:0								
PC 40:1			22:1	22:0	24:0								
PC 44:1	24:1												
PC 26:0													
PC 28:0			**10:0*****										
PC 30:0	**10:0*****		**12:0*****										
PC 32:0	**12:0*****	13:0	**14:0****										
PC 34:0	**14:0****	**15:0***	**16:0**										
PC 36:0	**16:0**	**17:0***	**18:0**										
PC 40:0	20:0		22:0										
PC 42:0**	22:0		24:0										

Table 18: Risk (2h), increased levels compared to baseline are highlighted in bold (red), decreased levels compared to baseline are highlighted in light grey (blue), and those with no significant changes black. In the middle boxes, those fatty acid species are shown from which the respective PC species could potentially be built with the corresponding LPC species (at the top). PC species are shown on the left. A significant decrease is defined as ≥ 1.2. Additionally, the significance we calculated with is shown in stars: *<0.05, **<0:01, ***<0.001, (scheme based on description by Thomas Kopf).

After four hours, PC 38:2 (S 30) was reduced in the risk group while the fatty acid 20:2, from which PC 38:2 could be built, was elevated. PC 36:5, which can contain 20:4 and is supposed to be protective against CVD [121] increased at 6 hours as a response to the OFTT, whereas FA 20:3 was also increased but concentration in the plasma of 20:4 did not change, indicating a withdrawal of FA 20:4 by the formation of PC 36:5.

	LPC 20:0	LPC 19:0	LPC 18:0	LPC 18:1	LPC 16:1	LPC 18:2	LPC 20:3	LPC 18:3	LPC 22:4	LPC 20:4	LPC 22:5	LPC 20:5	LPC 22:6
PC 40:7				22:6		22:5	20:4	22:4	18:3	**20:3**	18:2	20:2	18:1
PC 38:6				20:5	22:5	20:4	18:3	**20:3**		18:2	16:1	18:1	**16:0**
PC 40:6			22:6	22:5		22:4	**20:3**		18:2	20:2	18:1		**18:0**
PC 36:5					20:4	18:3		18:2	**14:1**	16:1	**14:0****	**16:0**	
PC 40:5	20:5		22:5	22:4			20:2		18:1		**18:0**	20:0	
PC 42:5	22:5									22:1	20:0	22:0	
PC 34:4					18:3		**14:1**	16:1	**12:0*****	**14:0****			
PC 36:4				18:3	**20:3**	18:2	16:1	18:1	**14:0****	**16:0**			
PC 38:4			20:4	**20:3**		20:2	18:1		**16:0**	**18:0**			
PC 40:4	20:4		22:4					22:1	**18:0**	20:0			
PC 42:4	22:4						22:1	24:1	20:0	22:0			
PC 32:3						**14:1**	**12:0*****	**14:0****					
PC 34:3					18:2	16:1	**14:0****	**16:0**					
PC 36:3			18:3	18:2	20:2	18:1	**16:0**	**18:0**					
PC 40:3	**20:3**					22:1	20:0	22:0					
PC 32:2				**14:1**	16:1	**14:0****							
PC 34:2				16:1	18:1	**16:0**							
PC 38:2	18:2		20:2		22:1	20:0							
PC 40:2	20:2			22:1	24:1	22:0							
PC 30:1				**12:0*****	**14:0****								
PC 32:1			**14:1**	**14:0****	**16:0**								
PC 34:1	**14:1**		16:1	**16:0**	**18:0**								
PC 36:1	16:1		18:1	**18:0**	20:0								
PC 38:1	18:1			20:0	22:0								
PC 40:1			22:1	22:0	24:0								
PC 44:1	24:1												
PC 26:0													
PC 28:0			**10:0*****										
PC 30:0	**10:0*****		**12:0*****										
PC 32:0	**12:0*****	13:0	**14:0****										
PC 34:0	**14:0****	**15:0***	**16:0**										
PC 36:0	**16:0**	**17:0**	**18:0**										
PC 40:0	20:0		22:0										
PC 42:0	22:0		24:0										

Table 19: Metabolic syndrome (2h), increased levels compared to baseline are highlighted in bold (red), decreased levels compared to baseline are highlighted in light grey (blue), and those with no significant changes black. In the middle boxes, those fatty acid species are shown from which the respective PC species could potentially be built with the corresponding LPC species (at the top). PC species are shown on the left. A significant decrease is defined as ≥ 1.2. Additionally, the significance we calculated with is shown in stars: *<0.05, **<0:01, ***<0.001, (scheme based on description by Thomas Kopf).

In the metabolic syndrome group, the increase of fatty acids does not co-occur with a decrease in PC species. PC 26:0, which has already been described to be increased in obesity, increased after two hours. In the metabolic syndrome group, PC 32:2 increased like in the risk group, LPC 18:3 increased and fatty acid 14:0 also increased. Four hours after the OFTT (S 33), the LPC species 18:1, 18:2, 18:3, 20:4 and 22:6 showed increased levels. PC 32:0 level, having the strongest association with mortality [121], did not show any remarkable change over time in any of the groups, even though the fatty acid species that this PC species contains were upregulated in all the groups after the OFTT.

3.4 Inflammatory markers in the blood compartment

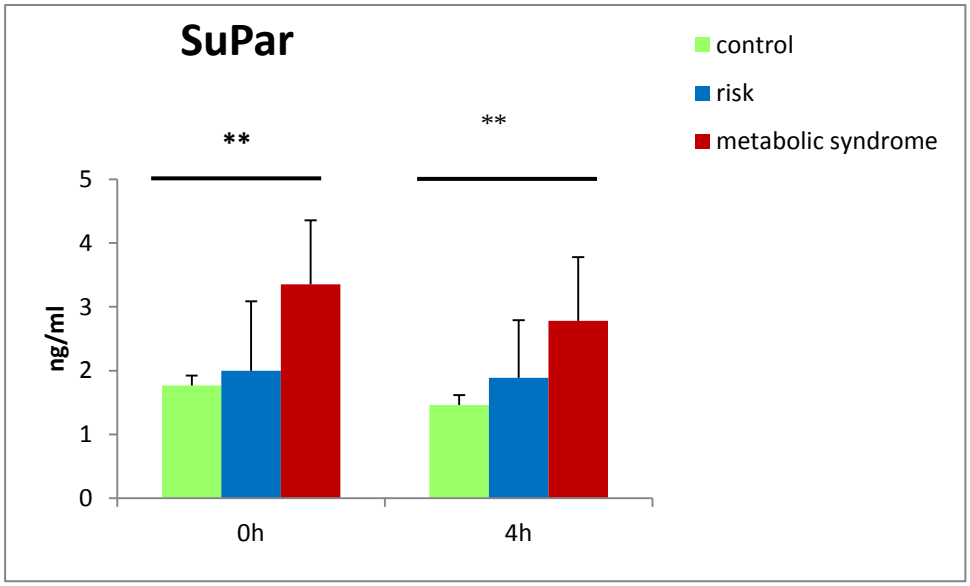
Inflammation markers were elevated in metabolic syndrome subjects. Although all the concentrations of inflammation markers changed during the application of the oral fat tolerance test, we found CRP to be the best inflammation marker for identifying the metabolic syndrome subjects. Metabolic syndrome subjects are in a state of chronic inflammation [122]. Therefore, we searched for differences in the time levels of well-established inflammation parameters using the oral fat tolerance test (OFTT). The inflammation parameters under investigation were CRP, the leukocyte counts and soluble urokinase-type plasminogen activator receptor (suPAR).

The urokinase-type plasminogen activator receptor (uPAR), which can be segregated into soluble uPAR (suPAR), is involved in immune activation and present in several cell types [123]. It plays a role in plasmin activation, modulation of cell adhesion, migration and proliferation, tissue remodeling and systemic inflammation. suPAR is generated in inflammatory response and is elevated in type II diabetes [123]:

"The urokinase plasminogen activator receptor (uPAR), which is present in several cell types , plays a role in numerous physiological pathways mainly involving immune activation, e.g. the plasminogen activating pathway, the modulation of cell adhesion, migration and proliferation, and tissue remodeling and systemic inflammation."[123]. uPAR is cleaved and released from cells in response to inflammatory stimulation, this leads to generation of suPAR [123]. SuPar can be regarded as a pro-inflammatory marker, thus providing a risk marker for type 2 diabetes [123]. A few studies have shown that, after adjustment for conventional risk factors, increased levels of suPAR in the general population are associated with an increased risk of death from all causes, as well as increased risk of several chronic diseases, including cancer, cardiovascular disease and type 2 diabetes" [123].

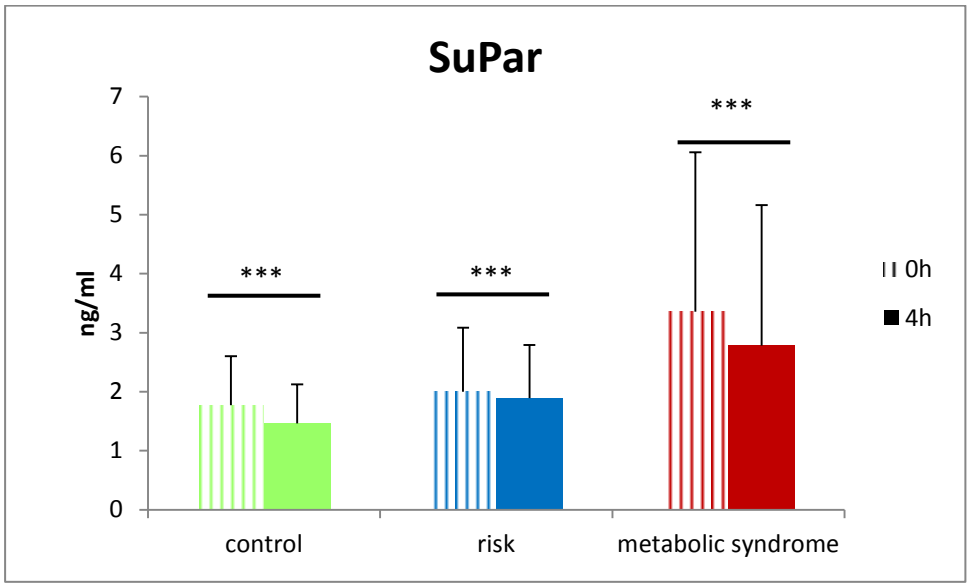
SuPar is known to be a predictor for CVD [124], and the results showed that basal suPar levels were significantly higher in the metabolic syndrome subjects than in the other groups (Figure 52 A). After the oral fat tolerance test, the values decreased in all three groups (Figure 52 B): Friedman test, $p<0.001$.

A



n=14(control); n=19 (risk); n=29 (metabolic syndrome), p<0.05= *; p <0.01=**; p<0.001=***

B



n=14 (control); n=19 (rik); n=29 (metabolic syndrome), p<0.05=*; p<0.01=**; p<0.001=***

Figure 52 A) Comparison of the SuPar distribution in the groups at the start of the experiment and at 4 hours (p<0.05 = *; p<0.01 = **; p<0.001 = ***). Data is represented as means ± 1SE B) Comparison of the SuPar concentrations over time (p<0.05= *; <0.01=**;<0.001=***. Data is represented as means ± 1SD.

Furthermore, the leukocyte count was significantly lower in the control group compared to the metabolic syndrome group. To compare the leukocyte number between the groups, the Kruskal-Wallis test was used (p=0.03). The leukocyte concentration was corrected by the dilution factor as explained in the "Material and Method" section. In order to compare the leukocyte concentration over time, Friedman was used (p<0.001 control, n=11; p<0.001, risk, n=19; p=0.02, metabolic syndrome; n=22). Levels changed significantly over time for all three inflammation markers.

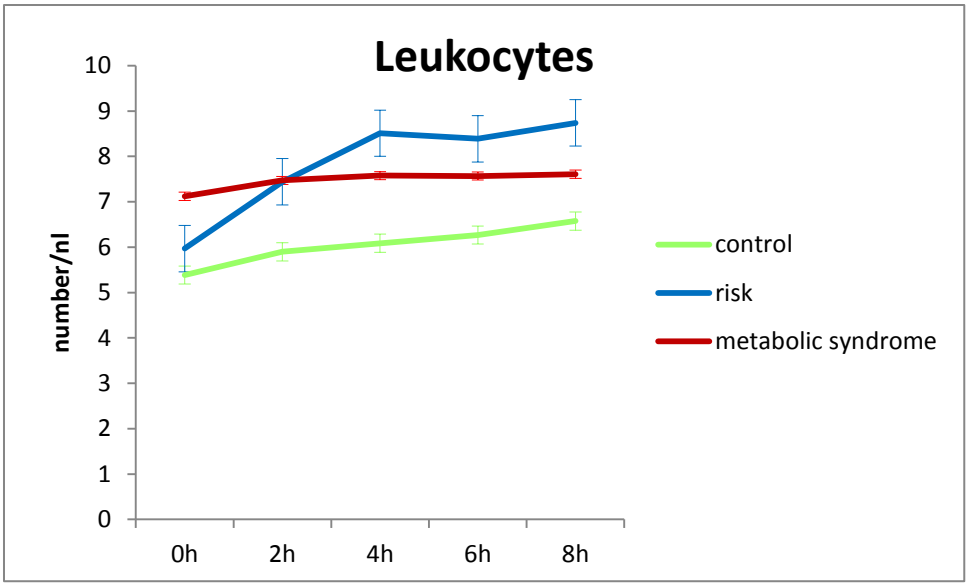


Figure 53: Representation of the leukocyte concentrations, corrected by dilution factor. The data was represented in means ± 1SE. Distribution between the groups was compared using Kruskal-Wallis, n=67, and Mann-Whitney U. Changes over time were tested by Friedman.

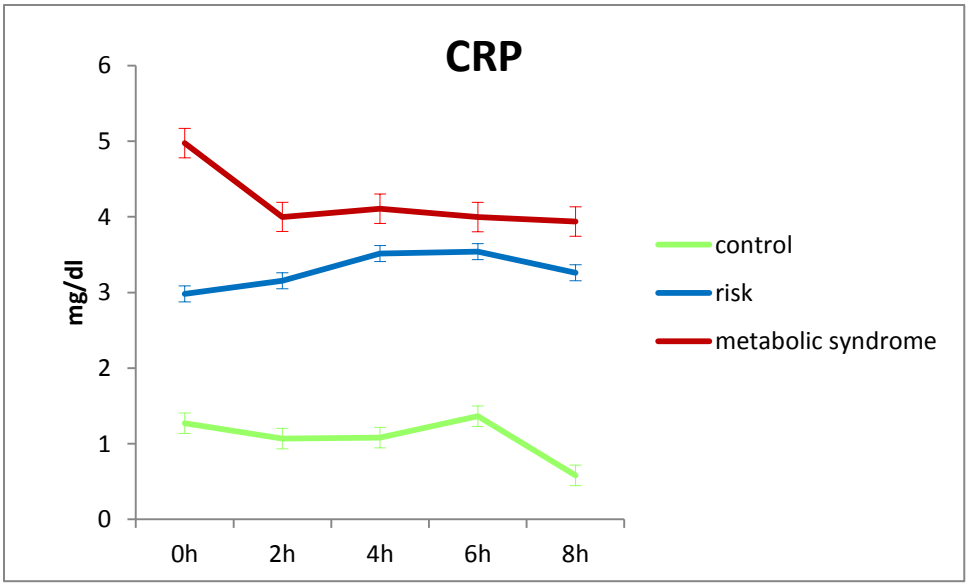


Figure 54: Representation of CRP n=13 (control); n= 20 (risk), n=33 (metabolic syndrome).
There was a significant difference in CRP concentration between the groups. The changes over time were significantly different in the metabolic syndrome group. The data was presented in means ± 1SE. Distribution between the groups was compared using Kruskal-Wallis and Mann-Whitney U. Changes over time were tested by Friedman.

CRP exhibited different kinetics over time in the different groups (Figure 54). Interestingly, the metabolic syndrome group showed decreases in CRP (Friedman test, p=0.027), while the levels of the other groups remained constant (p=0.291, control and p=0.658 risk). There also was a significant difference in CRP concentration between the groups (Kruskal-Wallis test, p=0.003). Summing up, levels of SuPar (Figure 52), CRP (Figure 54) and leukocyte counts

(Figure 53) were significantly lower in the control group compared to metabolic syndrome subjects and showed an acute response to the OFTT. The CRP concentration differed between the groups, reflecting chronic alteration of metabolism. CRP also reflects an acute alteration of the metabolism in metabolic syndrome subjects, manifested in a different reaction of the metabolic syndrome group to the drink compared to the other groups.

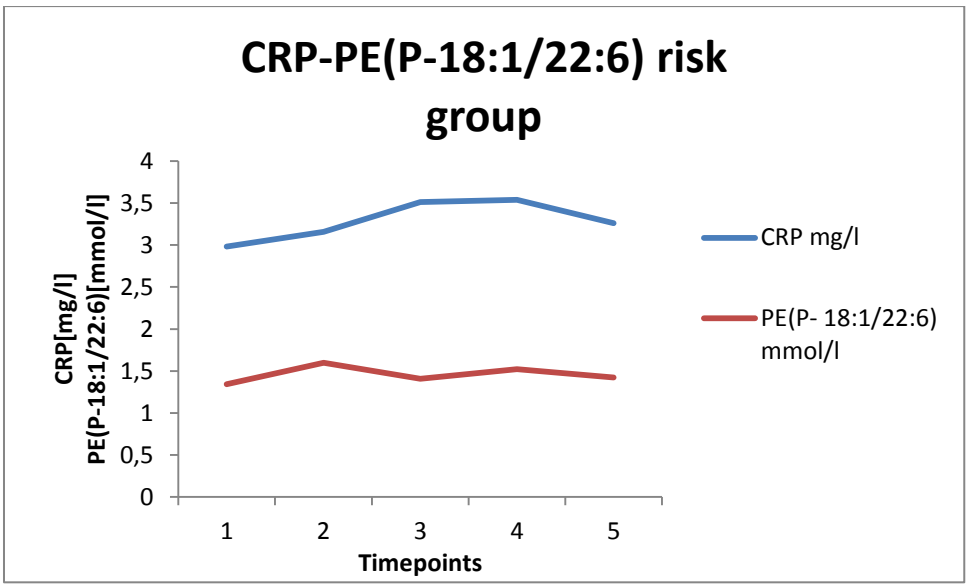


Figure 55: Dynamic over time of CRP and plasmalogen species 18:1/22:6 in the risk group

We found a negative correlation between CRP levels of the risk group (Figure 55) and the plasmalogen species 18:1/22:6 (correlation coefficient r = -0.63, risk). In the other groups there was no significant correlation (r = -0.05, control; r = 0.31 metabolic syndrome). The fatty acid docosahexaenoic acid (DHA) in sn-2 position has been reported to have an anti-inflammatory function. A decreased level of this fatty acid results in a decrease of all plasmalogens species [125]. The fact that only the risk group showed a significant negative correlation between this DHA-containing plasmalogen and CRP might be due to the small size of the groups.

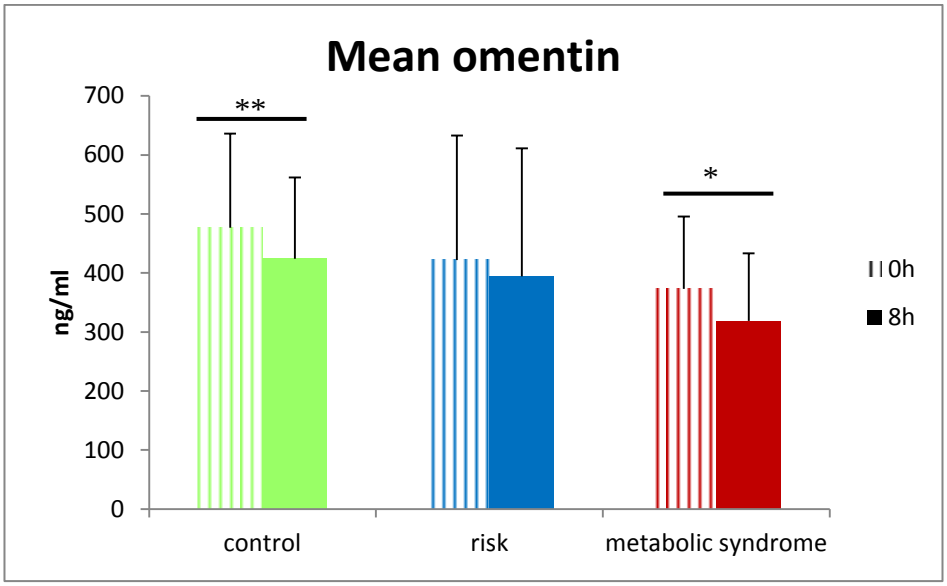
3.5 Adipose tissue hormones

The measurement of the adipokine levels of ghrelin and leptin after the OFTT enables the discrimination between subjects with risk for metabolic syndrome. Des-acyl-ghrelin [126], adiponectin[127] and omentin [128] are involved in the adipose tissue metabolism. In this study, omentin, adiponectin and ghrelin were analyzed as potential marker cytokines for the regulation of adipose tissue metabolism. We found that baseline levels of these adipokines were significantly lower in metabolic syndrome subjects compared to controls, indicating

chronic long-term adaptations of the adipose tissue in metabolic syndrome. As a reaction to the oral fat challenge, we found delayed and – in some cases – diminished reactions of the metabolic syndrome group compared to the others, which indicates an acute change in metabolic reaction to fat load.

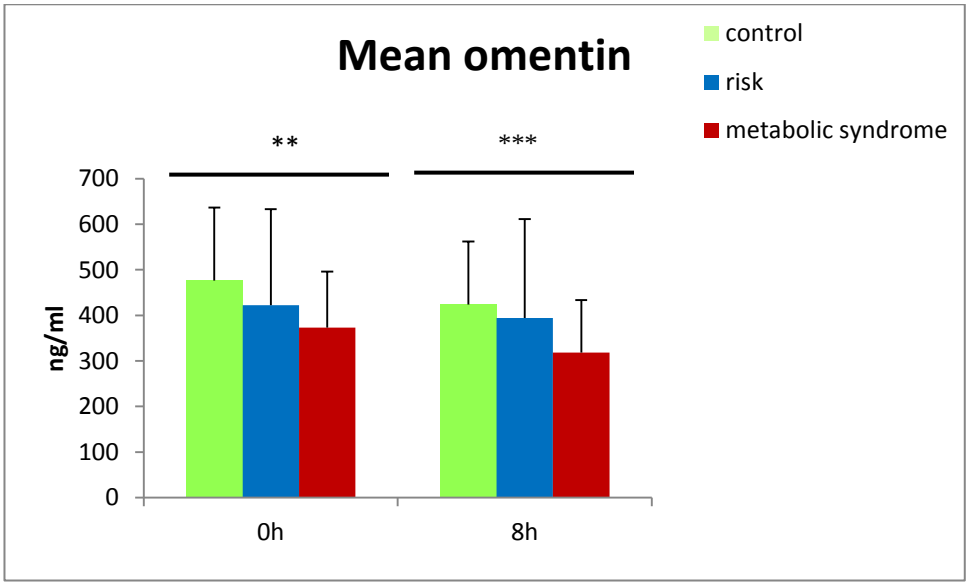
3.5.1 Omentin

A



n=14 (control); n=18 (risk) ;n=28 (metabolic syndrome)

B



n=14(control); n=18 (risk) ; n=28 (metabolic syndrome)

Figure 56 A) Comparison of **omentin distribution** in the groups at the start of the experiment and at 8 hours ($p<0.05$ = *; $p<0.01$=**; $p<0.001$=***) B) Comparison of omentin concentrations over time ($p<0.05$= *; $p<0.01$=**; $p<0.001$=*** The data are represented as means±1SE. Distribution between the groups were compared using Kruskal-Wallis and Mann-Whitney U. Changes over time were tested by Friedman.

Omentin is a cytokine which modulates inflammation [129]. Omentin levels were found to be decreased in metabolic syndrome subjects compared to control subjects [129]. This study (Figure 56) confirmed the decreased basic omentin values of the metabolic syndrome subjects [130] (Mann-Whitney U, p=0.013). Moreover, the results showed a significant response to the oral fat tolerance test in control (Friedman, p=0.002) and metabolic syndrome subjects (Friedman, p=0.016). The risk subjects also showed the same trend, but this result was not statistically significant (p=0.059). Since reactions to the oral fat tolerance test in the groups were the same, the oral fat tolerance test did not exhibit discriminatory potential for identifying subjects from the respective groups (Figure 56).

3.5.2 Leptin

"Leptin is involved in various physiological processes. It derives mostly from adipocytes, but it is also produced by immune cells." [131] Under physiological conditions, the amount of leptin produced by the adipose tissue (AT) is related to its mass [131]. "Shortly after food intake, leptin levels rise, interacting with its receptors in the hypothalamus, being followed by a decreased food intake and increased energy expenditure. Leptin also seems to be a critical mediator in the host defense during infections, stimulating innate responses as well." [131]

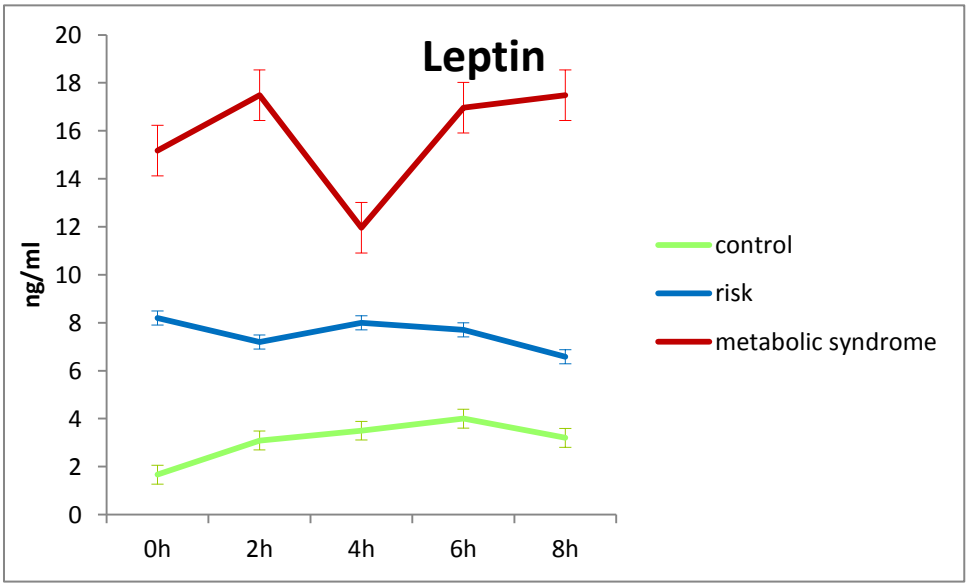


Figure 57: Leptin concentrations. Comparison between the groups was calculated by Kruskal-Wallis and Mann-Whitney U, p***, n=10 (control); n=19 (risk); n=31 (metabolic syndrome). Over time, the changes were calculated using the Friedman test n=3, (control); n=10 (risk); n=19 (metabolic syndrome). The changes over time were significantly different in the metabolic syndrome and the risk group. The data is represented as means±1SE.

Basal leptin values were significantly higher in risk and metabolic syndrome subjects (Figure 57) compared to control subjects (Mann-Whitney U test, $p<0.001$). Each group, as compared to the others, showed a significant difference. Over time, the values of the control subjects remained constant (Friedman, $p=0.066$, control, $n=3$), while the risk and metabolic syndrome subjects showed a significant change (Friedman, $p=0.001$, risk; $n=10$; $p<0.001$, metabolic syndrome, $n=19$). After the oral fat tolerance test, metabolic syndrome subjects had their nadirs at 4 hours, whereas the other groups did not show this significant decrease. Since serum half-life of leptin is 90 minutes, its elimination was obviously delayed in metabolic syndrome subjects, or its production was increased. The risk group showed a nadir at 120 minutes, which was not significant. The oral fat tolerance test provided additional information on an acute adapted metabolism in the metabolic syndrome subjects. When comparing those adipokines with the lipids, we observed that leptin correlates positively with the palmitoleic acid 16:1 in the risk group.

3.5.3 Adiponectin

As a next step, we measured the adiponectin concentration using ELISA. Adiponectin is an insulin-sensitizing adipokine as well as an anti-inflammatory and antiatherogenic hormone [132].

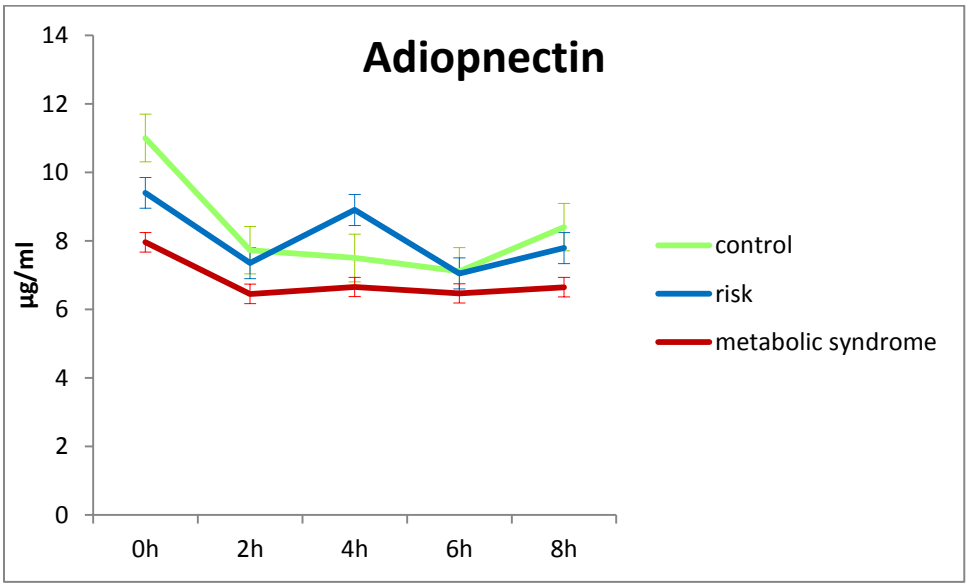


Figure 58 : The mean value of adiponectin concentrations between the groups was calculated by Mann-Whitney U. All points in time included n= 75(control), n=98(risk) n=159(metabolic syndrome.). Over time, there was a response to the OFTT in the control and risk groups. Data is represented as means ± 1SE.

When we compared the control group to the metabolic syndrome group (Figure 58) using the Mann-Whitney U test by considering all points in time simultaneously, we found a basal difference between the control group and the metabolic syndrome group ($p<0.001$) as well as between the control and the risk groups ($p=0.006$). There was no significant difference between the risk and the metabolic syndrome groups ($p= 0.114$). However, mean adiponectin levels were higher in the control group than in the risk group and mean adiponectin levels in the risk group were higher than in the metabolic syndrome group. As a reaction to the drink, there was a response over time in the control group ($p<0.01$) and the risk group ($p<0.01$), whereas there was no significant change over time in the metabolic syndrome group ($p=0.056$). The behavior of the groups over time was similar: at two and six hours all groups reached their nadirs. At 4 hours there was a peak in each group, but these peak levels still remained statistically lower than the basal levels of the groups.

3.5.4 Leptin-adiponectin ratio

Earlier in this thesis, the leptin-adiponectin ratio has been described to be a more sensitive predictor for CVD than adiponectin and leptin alone [133]. Metabolic syndrome subjects, as shown in Figure 59, had a higher leptin-adiponectin ratio than the other groups as an indication of an elevated risk of CVD.

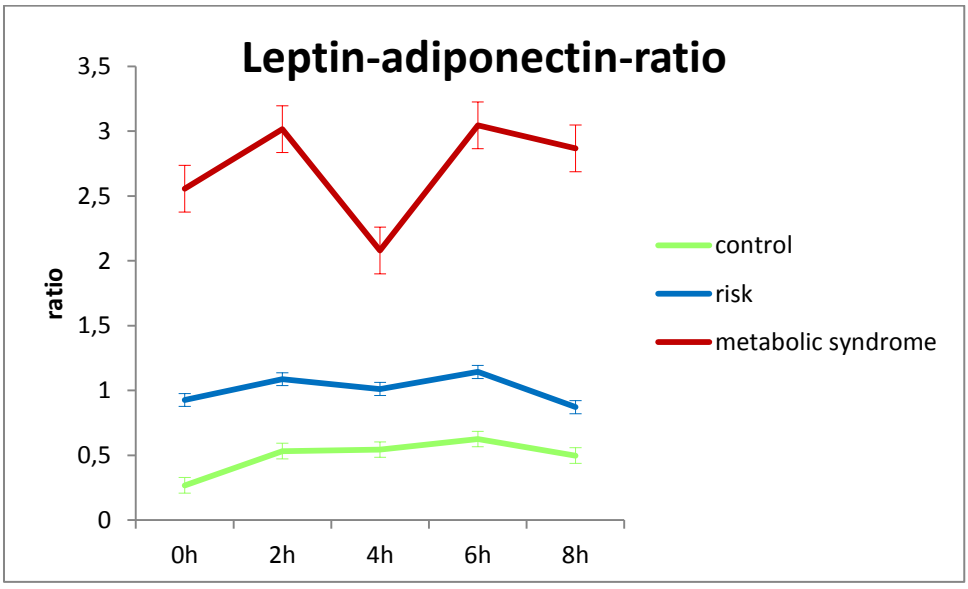


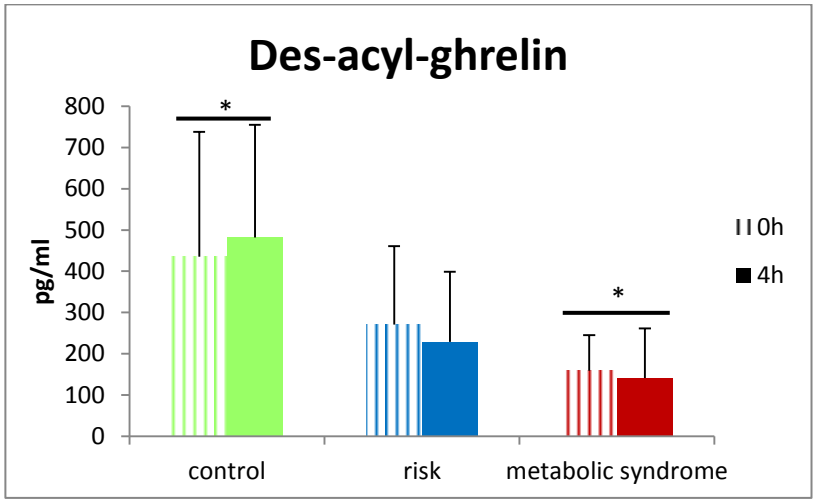
Figure 59: The comparison of the adiponectin-leptin ratio between the groups was tested by Mann-Whitney U (control-metabolic syndrome);(control-risk); (risk-metabolic syndrome). The adiponectin-leptin ratio showed a significant difference at time = 0 hours between the groups. Over time, there was a significant difference in the risk and the metabolic syndrome groups. Significance of the changes over time tested using Friedman test n=3 (control); n=10 (risk); n=20 (metabolic syndrome). Data is represented as means ± 1SE.

The adiponectin-leptin ratio showed a significant difference at time = 0 hours between the groups (Mann-Whitney U, p<0.001 control-metabolic syndrome; p=0.016 control-risk; p= 0.047 risk-metabolic syndrome). Over time, there was a significant difference in the risk and the metabolic syndrome groups (Friedman test, p=0.316, control, n=3; p<0.001, risk, n=10; p<0.001, metabolic syndrome, n=20). The results show that the metabolic syndrome and the risk groups showed a significant reaction to the drink, whereas the control group did not react. The ratio may reflect not only the chronic, but also the acute adaptation of the metabolism in metabolic syndrome subjects and also in the risk group. The adiponectin-leptin ratio could be used as a marker to identify metabolic syndrome subjects in prevention screenings.

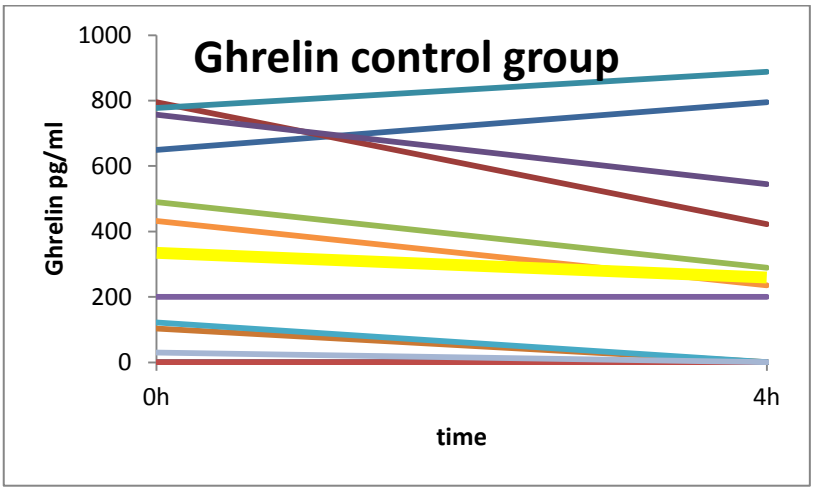
3.6 Gastrointestinal tract: Ghrelin

Ghrelin is a cytokine that is secreted mainly by the gastrointestinal tract but also by the fatty tissue [134] and that is involved in inflammation [135].

A

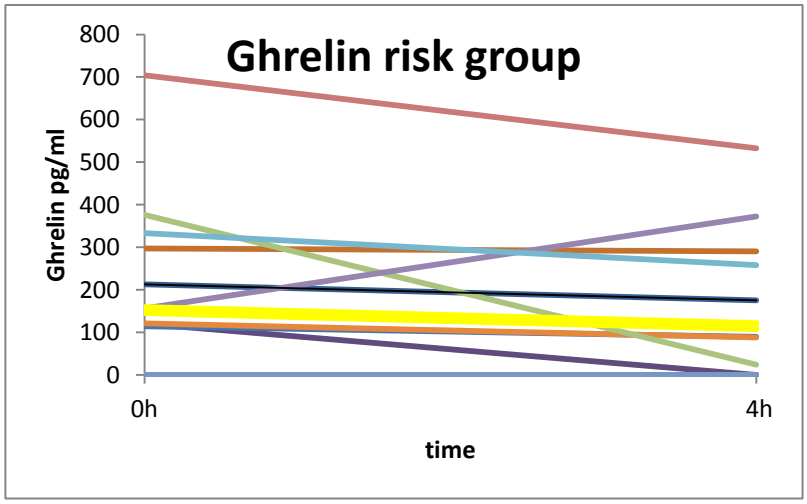


B



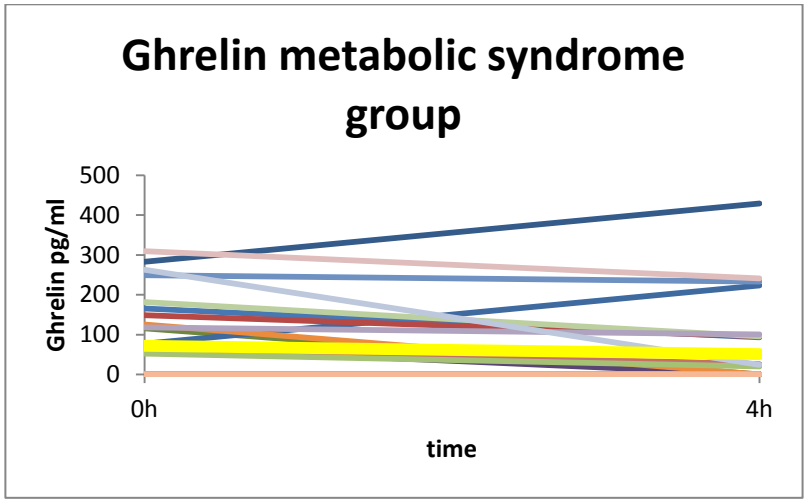
control n=13

C



Risk, n=16

D



Metabolic syndrome, n=31

Figure 60, A-D: Ghrelin. A) Comparison of the ghrelin distribution over time (t=0 and t=4hours) (p<0.05= *; <0.01=**;<0.001=***); Control; n=7, risk; n=8, metabolic syndrome n=11. B) Changes over time of the ghrelin levels in the control group, including values below detection limit; C) Changes over time of the ghrelin levels in the risk group, including values below detection limit; D) Changes over time of the ghrelin levels in the metabolic syndrome group, including values below detection limit. The data is represented as means ± 1SD, distribution between the groups was compared using Kruskal-Wallis and Mann-Whitney U. Changes over time were tested by Friedman test. The mean is represented as a yellow line in B,C and D.

The mean ghrelin concentration (Figure 60 A) showed significant differences between the control and the metabolic syndrome groups (Mann-Whitney U, p=0.048). The significance was calculated using the Mann-Whitney U test. The comparison of the other groups showed no significant differences (p=0.1, control–risk; p=0.3 risk-metabolic syndrome). At different points in time, there was a significant difference within the risk and the metabolic syndrome groups (Friedman test, p=0.414, control, n=7; p=0.034, risk, n=8; p=0.035, metabolic syndrome, n=11). In order to increase the number of cases, graphs B, C and D in Figure 60 were created, which also include the cases with values below detection limit. For the purpose of those diagrams, as described in the "Material and Method" section, the values below

detection limit were set at 0.2. The result of the significance test was similar to previous results. Thus, the control group (Figure 60 B) showed no changes over time (p=0.096), the risk (Figure 60 C) and the metabolic syndrome (Figure 60 D) groups showed significant changes as a reaction to the drink (p=0.02 and p=0.008). In the final analysis, basal levels of ghrelin were significantly lower in subjects with metabolic syndrome (Figure 60 D) than in control subjects (Figure 60 B) and showed a significant decrease four hours after the fat tolerance test in the metabolic syndrome (Figure 60 D) and the risk subjects (Figure 60 C). In the control group (Figure 60 B), there was no significant response to the drink.

3.7 Renal system

3.7.1 Creatinine

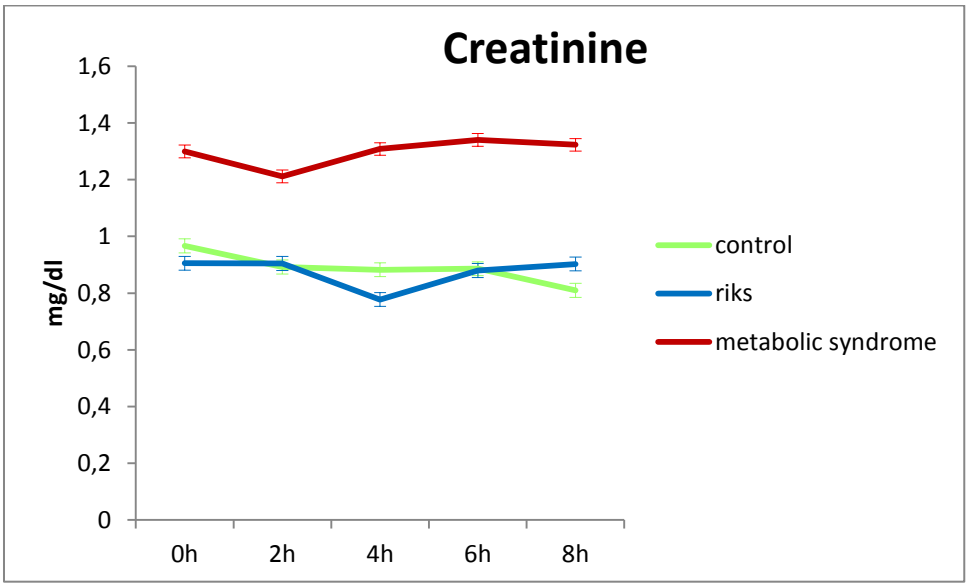


Figure 61: The comparison of creatinine concentrations was calculated by Kruskal-Wallis n=13 (control); n=20 (risk) , n=33 (metabolic syndrome). There was a significant difference between the groups at baseline. Changes over time were calculated using the Friedman test: (control; n=3); (risk; n=7); (metabolic syndrome; n=17). Data is represented as means ± 1SE

The creatinine concentration (Figure 61) did not change significantly over time in any of the groups (Friedman test, p=0.087, control, n=3; p=0.439, risk, n=7; p=0.596, metabolic syndrome, n=17). However, basal values of the metabolic syndrome subjects were chronically elevated, considering all points in time at once (Kruskal-Wallis, p= 0.002). In order to show that the kidney function was also impaired in those metabolic syndrome subjects without diabetes, we correlated kidney function with diabetes (Figure 62). A reduced kidney function was defined by elevated creatinine levels (>1.1 mg/dl) at any point in time.

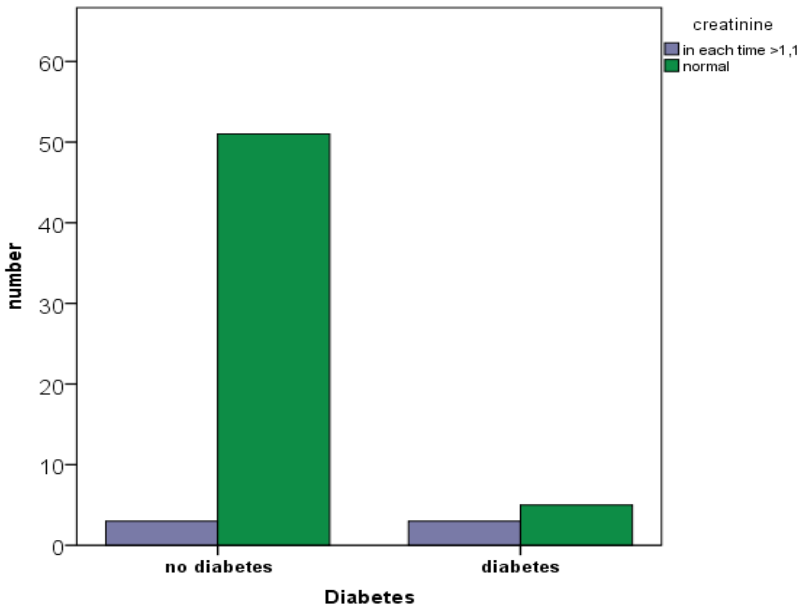


Figure 62: Diabetes and kidney function.

3.7.2 FGF-23

FGF-23 is a fibroblast growth factor which acts as a hormone and is produced by osteoblasts/osteocytes in the bone. It acts on the kidney by regulating the phosphate and vitamin D metabolism through activation of the FGF receptor/α-Klotho co-receptor complexes [136]. It is possible that elevated FGF-23 may exert its negative impact through distinct mechanisms of action independent of its role as a regulator of phosphorus homeostasis [136]. Elevated circulating FGF-23 concentrations have been associated with left ventricular hypertrophy (LVH), and it has been suggested that FGF-23 exerts a direct effect on the myocardium [136]. While it is possible that 'off-target' effects of FGF-23 present in very high concentrations could induce LVH, this possibility is controversial, since α-klotho is not expressed in the myocardium. Another possibility is that FGF-23's effect on the heart is mediated indirectly, via 'on-target' activation of other humoral pathways [136].

We found significant chronically ($p<0.001$) and acutely elevated FGF-23 levels in patients with metabolic syndrome compared to the other groups (Figure 63). The acute reaction to the oral fat challenge in the metabolic syndrome subjects ($p<0.001$) and constant FGF-23 levels in healthy subjects renders FGF-23 a suitable biomarker for the metabolic syndrome. The acute elevation after the drink could be due to a reduced clearance capacity in metabolic syndrome subjects. This reduced clearance capacity could again be interpreted as a sign of resistant metabolism in the metabolic syndrome subjects.

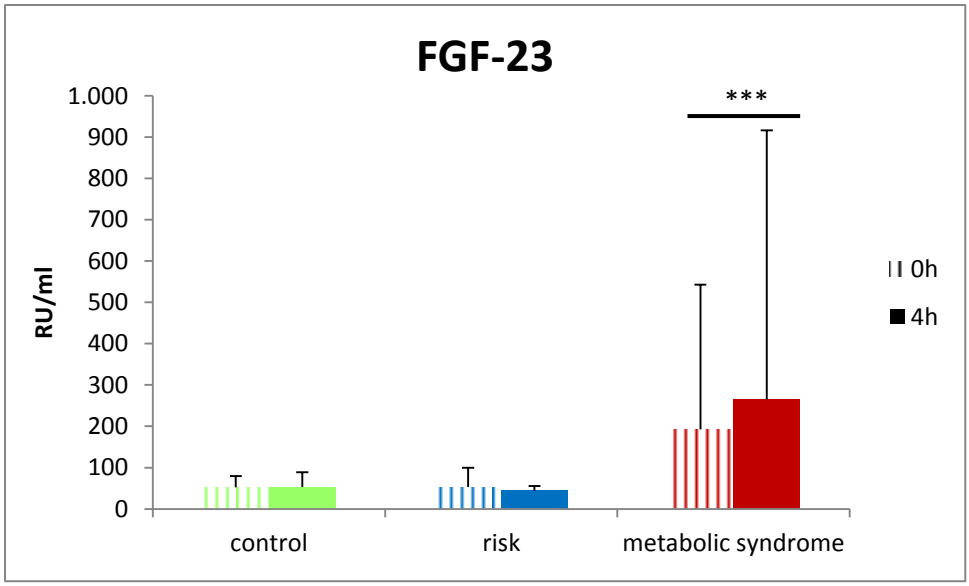


Figure 63: FGF-23 concentrations. Comparison of the FGF distribution between the groups at the start of the experiment and at 4 hours, tested by Kruskal-Wallis. FGF-23 concentrations over time showed a significant difference in the metabolic syndrome group, tested by Friedman test: $p<0.001$ (The data are presented as means ± 1SD, distribution between the groups were compared using Kruskal-Wallis and Mann-Whitney U.Changes over time were tested by Friedman test, $p<0.05$= *; $p<0.01$=**; $p<0.001$=***).

To clarify whether the elevated FGF-23 level in the metabolic syndrome subjects is only an accompanying parameter of the reduced kidney function or whether it is independent of kidney function and a consequence of the metabolic syndrome, the FGF-23 levels of non-diabetic subjects with normal kidney function were selected and shown in Figure 64. In order to find out whether FGF-23 could be a biomarker for the metabolic syndrome subjects in general and independently of kidney function, the FGF-23 levels of the three groups were compared by only referring to selected subjects with normal kidney function and without diabetes. Since the number of non-diabetic subjects with intact kidney function was low at time zero, the standard error was too large. Therefore, all time-points were accumulated and the FGF-23 levels were then compared.

The chronic elevation of FGF-23 in healthy-kidney and non-diabetic metabolic syndrome subjects was significant at all points in time together (Kruskal-Wallis test, $p<0.001$, control, n=63, risk n=92, metabolic syndrome, n=82). The response to the drink was different when comparing the groups. Whereas the control and risk group did not show any significant changes over time, the metabolic syndrome group showed an increase of the FGF-23 levels as reaction to the oral fat tolerance challenge (Friedman, $p=0.147$, control, n=11; $p=0.205$, risk, n=18; $p=0.031$, metabolic syndrome, n=16). The values increased until 6 hours, afterwards they decreased again. Measuring FGF-23 was a method to prove an acutely altered

metabolism in metabolic syndrome subjects, so FGF-23 could be a new biomarker measured in non-fasting condition for metabolic syndrome. Further analysis with a larger group size could be useful for evaluating the benefit of fasting values.

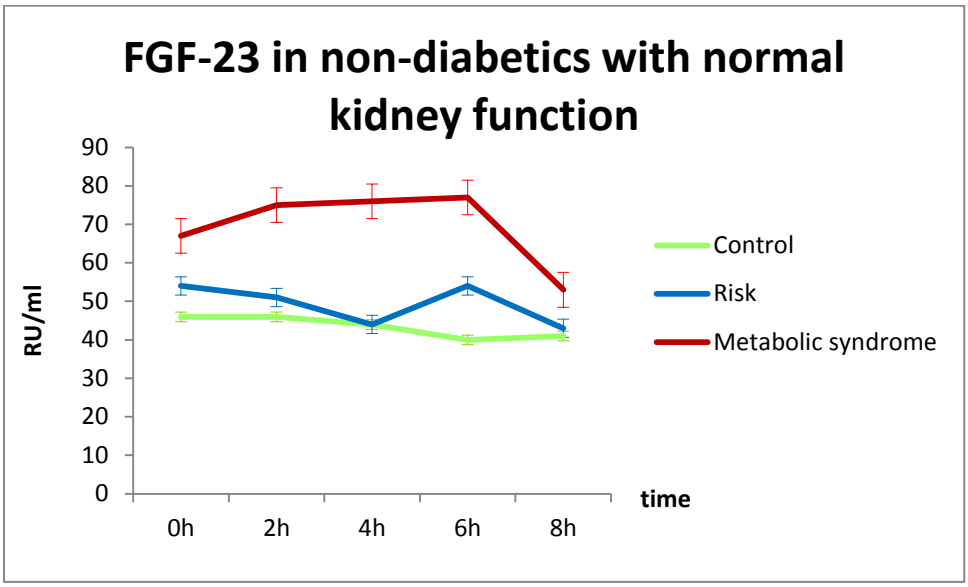


Figure 64: The FGF-23 concentration in the subjects with normal kidney function (creatinine <1,1 in at least one point in time) and without diabetes. Comparison between the groups was tested using Kruskal-Wallis including all points in time. n=13 (control) n=18 (risk) n=16 (metabolic syndrome). Changes over time were calculated using the Friedman test: n=11 (control); n=18(risk); n=16(metabolic syndrome). Over time, there was a response to the OFTT in the metabolic syndrome group. Data is represented as means ± 1SE.

4 Discussion

4.1 Accuracy of choice of stratification criteria

To find several biomarkers which in combination could help to classify subjects in a risk group for the metabolic syndrome early and provide early treatment, at first the 67 volunteers were classified in three groups according to IDF criteria as described above. The distribution of the individuals in the three groups control, risk and metabolic syndrome results in different group sizes. The metabolic syndrome group contains 33 subjects, whereas the control group contains only 14 subjects (Figure 5). We measured different parameters before and during the oral fat tolerance test (OFTT). The comparison of the mean concentrations showed differences between the groups. At first, the triglyceride and cholesterol concentrations, as well as the concentrations of their vehicles, were analyzed. Since the criteria for the metabolic syndrome [77] include TAG and HDL levels as stratification parameters, the groups showed significantly different HDL and TAG levels. Interestingly, we only found diabetes in the metabolic subjects group, although we did not – as suggested by the WHO [77] – include insulin resistance as a necessary criterion for the metabolic syndrome. Fasting glucose was significantly higher in the subjects with risk factors than in the control subjects, which was expected since normal glucose concentration was an inclusion criterion for control subjects. Hypertension occurred significantly more frequently in the metabolic syndrome group than in the risk group. In the control group, we did not have any subject with hypertension.

OFTT

The oral fat load consisted of 66% fat with mostly saturated fatty acids. A drink containing a bigger proportion of fat and less carbohydrate would be better suited for relating the effects to the fat challenge more easily. The contained glucose can distort the reaction to FA in insulin sensible subjects [137]. This could cause different reactions in the groups. On the other hand, we did not only add the sugar for the taste, but also because a pure fat load would fail to elicit the metabolic responses that depend on the increase in plasma-insulin concentrations. That is because insulin is a key agent for changes in adipose tissue, caused by fatty acid circulation, during the transition from fasting to fed state [138].

4.1.1 Lipid vehicles and other Cholesterol fractions

There was no significant difference in total cholesterol concentrations, non-HDL cholesterol concentration and LDL concentration between the groups. We had expected a higher LDL concentration in the metabolic syndrome group than in the control group. The results proved that with a probability of 90% there is no difference between the concentrations in the groups. This might be due to the small group sizes. But since no trend was recognizable, it can be assumed that the LDL values were in fact the same in all groups. But the treatment needs differ between the groups. The control group had none of the risk factors linked with atherosclerosis (impaired glucose tolerance, low HDL concentration, elevated TAG concentration, elevated body weight or blood pressure) and for primary prevention, it is not reasonable to treat subjects without risk factors and cholesterol values beneath 160 mg/dl [139]. Even the risk subjects with a mean LDL of 129 mg/dl are "near optimal" and do not exceed the threshold value for treatment of 130 mg/dl [139]. It should be considered to treat the metabolic syndrome subjects, since the target value for secondary prevention is 100 mg/dl [139]. Over time, there was no significant change of LDL concentration in any of the groups, but we observed a trend towards a decrease in LDL concentration in the control group without statistical significance. This is in line with previously published literature [140]. Another work group recently tested the responses of normo- and hypertriglyceridemic subjects to a high-fat meal. Their study revealed a decrease in LDL concentration between 0 and 6 hours following a high-fat meal in normotriglyceridemic men. In hypertriglyceridemic subjects, they found no change of LDL concentration over time [140]. Their study included a larger number of subjects and their shake consisted of a higher fat concentration, which adds validity to their study. In general, LDL is not a good predicting parameter for metabolic syndrome and CVD, which is why it is not included in the definition of the symptom complex of the metabolic syndrome [141,142]. A greater positive correlation between CVD and the apo B/apoA-I ratio has been proved previously [142].

"The ratio of apoB-100 to apoA-I reflects the balance of cholesterol transport in a simple way. The higher the value of the apoB-100/apoA-I ratio, the more cholesterol is likely to be deposited in the arterial wall, thereby provoking atherogenesis and hence also increasing CVD risk" [98].

We calculated the ratio, which was significantly higher in the metabolic syndrome subjects than in the control subjects. This result was in line with previous reports, which stated that the ratio is higher and associated with an elevated risk for CVD [143]. In summary, the metabolic

syndrome group showed a higher apoB-100/apoA-I ratio compared to the other groups, although there was no significant difference in basal LDL levels. The higher apoB-100/apoA-I ratio indicates a chronic metabolic adaptation of the metabolic syndrome group to the constantly high calorie load.

The constant HDL concentration over time diverged from previously published observations of a decrease in HDL concentration in a diabetic group with visceral obesity at 3 and 5 hours [144]. A study by Wojczynski et al. found a decrease of HDL in a control and a hyper-triglyceridemic collective of men 3 and 5 hours after an oral fat challenge [140]. The latter's fat drink contained 83% fat, as opposed to 66% in ours. Another work group observed a decrease of the HDL and LDL concentrations in a metabolic syndrome group in the LIPGENE study. Their study population consisted of 60 men and 120 women. Their parameters were tested after several fat load tests of different concentrations [145].

Atherogenic properties of HDL and LDL species

Other chronic metabolic adaptations were revealed by the sizes of the cholesterol transporter particles in relation to their atherogenity. Less atherogenic species showed higher concentrations in the control group, shown by the LDL/apoB-100 ratio and apo A-I/HDL-ratio we used to differentiate between size and number of LDL and HDL particles. The LDL/apoB-100 ratio was higher in healthy subjects than in risk and metabolic syndrome subjects. This corresponds to a previous publication which shows that a higher ratio of apoB-100/LDL is associated with the metabolic syndrome [146]. A higher ratio of LDL/apoB-100 is a sign of larger LDL particles, which means that they are less atherogenic [147]. To conclude, the control subjects had fewer and larger LDL particles, but the total amount of LDL was the same in all three groups. The apo A-I/HDL-ratio was lower in the control subjects than in the risk and the metabolic syndrome subjects. HDL levels were higher in the control subjects than in risk subjects and the apo A-I concentration did not show a significant difference between the groups. Large HDL particles have four copies of apo A-I, small HDL particles have two copies of apo A-I [100]. The lower ratio in the control group leads to the conclusion that the control group has smaller HDL particles than the metabolic syndrome group. This result does not correspond to previous publications [148]. But it is known that in healthy subjects, small HDL particles are the ones which possess protective mechanisms against atherosclerosis, whereas in the metabolic syndrome group this capacity is lost [149]. This supports our results. Moreover, the results indicated an increase in apo A-I containing HDL fractions and a failure to generate mature, cholesterol-rich HDL in the metabolic syndrome group. This can be

deduced from the fact that the premature discoid HDL contains primarily apo A-I [99]. Thus, the ratio may indicate a chronic metabolic adaptation in the metabolic syndrome subjects and could therefore serve as a biomarker for the risk of metabolic syndrome in fasting and postprandial conditions.

Lipid transport from the intestine and to the liver reflected by apoB-48/apo A-I

No data on the apoB-48/apo A-I ratio, which reflects the relation of the chylomicrons to apo A-I, has been published previously. Our results did not show a difference of this ratio between the groups. But in both the risk and the metabolic syndrome groups, an acutely altered metabolism was reflected by an acute response to the drink. Thus, in postprandial state, the apoB-48/apo A-I ratio can serve as biomarker for the overproduction and disturbed metabolic clearance of chylomicron remnant particles in the metabolic syndrome subjects.

4.2 Adaptation to metabolic syndrome by acute and chronic changes of the physiology in metabolic syndrome subjects

4.2.1 CVD association with postprandial state marked by acutely elevated-chylomicron concentration

Postprandial or fasting lipid profiling for risk evaluation of the metabolic syndrome

Diabetes can be a reason for hyper- and dyslipoproteinemia. Hyper- and dyslipoproteinemia are associated with an elevated risk for CVD. “Dyslipidemia and impaired glucose intolerance are associated with atherosclerosis and coronary heart disease” [150]. Under fasting conditions and non-fasting conditions, cardiovascular disease correlates with low HDL and high LDL levels. Several researchers have examined whether the postprandial lipid profile could be a more sensible indicator for the risk of cardiovascular disease than the fasting lipid profile and whether this could be applied to diabetic persons [151]. Their results support using non-fasting blood samples to assess dyslipidemia and CVD risk in diabetic and non-diabetic subjects [151]. Kolovou et al. recommend a single plasma TAG measurement four hours after an oral fat load [152]. In our study, we tried to achieve a valid statement about the connection between plasma lipid profile, metabolic syndrome and CVD risk, assuming that metabolic syndrome subjects have an elevated risk for CVD. We found out that the most sensitive

method of evaluating the CVD risk is by comparing the fasting lipid profile with the postprandial lipid profile. The importance of looking at the postprandial lipid profile has already been stated by Pang et al. His work group suggested that especially postprandial dyslipoproteinemia exacerbates inflammation and oxidative stress. This affects the walls of arteries, which is a risk factor for atherosclerosis and cardiovascular disease [153]. The metabolic syndrome subjects, whom we consider to always be in a postprandial state, have an elevated risk for CVD and an elevated TAG concentration. Metabolic syndrome subjects have a higher fasting VLDL than the control group but the same fasting apoB-48 concentration as healthy subjects. The apoB-48 concentration shows an acute increase in both the metabolic syndrome and the control groups after the oral fat tolerance test, but over time we observed a slower decrease in apoB-48 concentration in the metabolic syndrome subjects. The application of an OFTT resulted in a diminished increase in the serum apoB-48 concentration in the metabolic syndrome group compared to the increase in the control group. This indicates that metabolic syndrome patients are not able to reach a proper fasting state anymore but remain in a constant postprandial response. It also shows an "absorption resistance" (malabsorption syndrome) in metabolic syndrome subjects. ApoB-48 is already known as an exclusive marker of the number of circulating particles of chylomicrons and their remnants [154] and is hence the most specific postprandial marker whose turnover can also be determined with a stable isotopically labeled isotopomer [153]. In the metabolic syndrome subjects, insulin resistance might be a cause for the dyslipoproteinemia. Reyes-Soffer et al. state in their article: "measures of postprandial lipoproteins are not associated with CAD in patients with diabetes 2" and only apoB-48 would be a useful marker for CAD (coronary artery disease) risk in persons with diabetes [155]. We assume that it would be more suitable for determining the risk for CVD no matter whether individuals are diabetic or not. Thus, we aimed to find a difference in lipid profiles of healthy subjects and subjects with the risk of CVD, assuming that diabetes belongs to the risk factors for CVD and that the full risk spectrum is reached by the metabolic syndrome subjects.

Reasons for increased apoB-48 plasma levels in risk subjects

Chylomicrons (CM) are lipoproteins that serve as a vehicle for the fatty acid transport from the intestine to the target tissue. In healthy subjects, an acute fatty acid supply raises the concentration of chylomicrons and the risk for CVD [156]. The insulin resistance is jointly responsible for increased enterocyte production of chylomicrons, and the latter play a role in the induction of dyslipoproteinemia, like in a circulus vitiosus [153]. A bigger offer of

chylomicrons indicates a bigger amount of intestinal absorption of TAGs. In their article "Postprandial dyslipidemia and diabetes: mechanistic and therapeutic aspects", Pang et al. question whether the postprandial chylomicrons concentration can be a useful marker for CVD risk. Referring to this question, this study reveals different postprandial dynamics of chylomicron concentration over time comparing the plasma of individuals with an increased risk for CVD and control subjects. As an indicator for the chylomicron concentration, apoB-48, which is secreted by the intestine, was measured in plasma. An elevated apoB-48 plasma concentration indicates an increased chylomicron production in humans. The liver only secretes apoB-100 whereas the small intestine only secretes apoB-48 [157]. ApoB-48 is an essential constituent of chylomicrons, which transport TAGs through the plasma and could be measured and used as markers of exogenous TAG absorption via the intestine. Chylomicron secretion and size correlates with the present TAG concentration [153]. Type 2 diabetes is associated with an increased secretion rate of apoB-48, but also with an impaired clearance capacity. This leads to an elevated concentration of apoB-48 in plasma. This is firstly because the secretion is not stopped by insulin and secondly because the free FAs resulting from a diminished tissue uptake of FAs trigger apoB-48 secretion, while the clearance capacity of the lipolysis pathway is disturbed. Insulin suppresses lipolysis since it is anabolic and lipolysis is therefore elevated in diabetic subjects. The fatty acid spillover in the plasma which is due to diminished uptake and increased lipolysis in diabetics is responsible for higher apoB-48-levels [156]. ApoB-48 clearance competes with VLDL clearance and thus apoB-48 not only positively correlates with chylomicron levels but also with the VLDL concentration. The adiponectin concentration, which is reduced in the metabolic syndrome group, correlates with VLDL clearance since the LPL activity in the adipose tissue is lower in persons with low adiponectin [153,158]. However, the VLDL concentration is elevated in the metabolic syndrome subjects with a lower adiponectin concentration. The endogenous TAG secretion is also elevated in insulin resistance because of the diminished FA uptake, resulting in an FA spillover. This experiment shows via apoB-48 that after a high fat load, chylomicron secretion is elevated in all of the groups but extremely so in the metabolic syndrome group. Combining this result with the observation that those fatty acids which are resorbed via chylomicrons are those which show different reactions to the OFTT due to the group they belong to, whereas those which are independent of chylomicrons show no different reactions in the three groups, we assumed that the disturbance of the chylomicron metabolism is associated with the longer half-life of the lipotoxic fatty acids in the plasma compartment.

Comparison of the behavior of the long-chain fatty acids and their transporters (chylomicrons)

The examined medium-chain FAs (FA 10:0 and FA 12:0), which are absorbed without chylomicrons, show exactly the same behavior in all of the groups. For the long-chain fatty acids, which we will discuss in detail later – such as the polyunsaturated γ-linolenic acid – the metabolic syndrome subjects showed a different dynamic compared to the control group. In this work, not all the differences of fatty acid dynamics are presented, but a focus is put on those which could serve as potential biomarkers.

Need of elevated fasting apoB-48 in risk subjects deduced by their chronic postprandial state

Referring again to apoB-48, the basal apoB-48-level should also be elevated if these individuals are in a chronically fed state and apoB-48 serves, as stated in recent articles by Pang et el., as a maker for the fed state. I suggest two reasons why our plasma levels of apo-B-48 were basally not significantly different between the groups. We could see a difference, but it was not significant. This might have been due 1.to the small size of groups and due to 2. the low basal values of apoB-48, which lay in the 4µl range. At such low values and with a standard deviation of 2 µl, a significant basal difference might not be seen, even if it exists. In other sources, a basal difference of apoB-48 is described [156]. Whereas free fatty acids are released from circulating TAGs by LPL in fasting state [159], postprandially the lipolysis is suppressed by insulin in adipose tissue and the exogenous fatty acids are transported into the plasma by chylomicrons.

4.2.2 Liver: Chronically elevated VLDL secretion due to elevated TAG production

An adipose tissue which does not efficiently absorb fatty acids can be the cause for an oversecretion of TAGs by the liver. Under such conditions, VLDL, which brings the TAGs from the liver to its target tissue, is also overproduced. The overspill of VLDL is associated with elevated chylomicron levels as chylomicrons compete with VLDL for clearance pathways, regulated by LPL [160]. CMs are formed postprandially, whereas VLDLs are formed in the fasting state [157]. In order to compare the fasting values, VLDL is therefore more suitable than chylomicron levels [156]. This was confirmed in our study. Next, the VLDL concentration over time was examined. Recent studies have shown that the four-hour values are sufficient to see the maximum postprandial VLDL production, which does not

change four hours after an oral fat load neither in healthy nor in metabolic syndrome subjects [161]. The VLDL concentration has previously been examined after application of an oral fat meal over time in other studies. These were not able to show a reaction to the oral fat tolerance test either in healthy or in metabolic syndrome subjects [155]. Similarly, our results did not show a significant change in the VLDL concentrations in any group over time. Summing up, we can say that apoB-48 is a marker for acute change, whereas VLDL is a marker for a chronically fed state. This is due to the fact that the secretion of VLDL is a longer process since the FAs first have to reach the liver to be taken up by VLDL. The metabolic syndrome subjects are constantly in a postprandial state; this is again demonstrated by the high VLDL levels in metabolic syndrome subjects, which are a sign of a constantly elevated fatty acid transfer to the liver and of a constantly elevated apoB-100 secretion. But the elevated VLDL-level is also a sign of reduced degradation due to the insulin resistance. VLDL could be used either in a fasting or in a postprandial state as a marker for chronic changes; the apoB-48 concentration should be measured postprandially to evaluate acute changes in the metabolism. ApoB-100 and VLDL are related to familial combined HLP [162], the most frequent form of hyperlipidemia [163] in type 2 diabetes [162].

4.2.3 Fatty acids: Comparison of the baseline levels, dynamic over time and transformation catalyzing enzymes of the cytotoxic and protecting fatty acids

Fatty acids as established marker of postprandial state

As mentioned above, the medium-chain fatty acids that are not chylomicron-dependent show no significant differences between the groups. The subsequent analysis of the chylomicron-dependent fatty acids demonstrates differences between the basal levels of the three groups and in response to the OFTT. FA 17:0 cannot be built by carbohydrate, even when saturated. It is known as a marker for oral fat load [164]. This fatty acid showed an elevated concentration under fasting conditions in the metabolic syndrome group. This result indicates that those subjects do not reach a fasting state. In the risk group, the fasting value is also higher than in the control group.

Dynamic over time of the pro- and anti-inflammatory fatty acids

The thesis of an acute change of the metabolism was supported once again by the pro- and anti-inflammatory fatty acids. The main precursors of the pro- and anti-inflammatory fatty

acids γ-linolenic, arachidonic acid (AA), docosahexaenoic (DHA) and eicosapentaenoic (EPA) showed differences in risk and metabolic syndrome subjects over time, whereas the control group did not show any reaction to the OFTT. This makes them a new potential biomarker for the risk of metabolic syndrome. For eicosapentaenoic acid (20:5-c5, c8, c11, c14, c17), one of the protective omega-3 fatty acids, plasma levels in humans have not been examined after acute fat administration over time yet, apart from EPA administration itself. The reason for the increase over time in the metabolic syndrome group is not clear. Since in all groups the content of the drink is the same, this difference after the drink can be due to different absorption by the intestine, different intake by the tissues, decomposition and conversion by desaturases. Desaturases and elongases are involved in maintaining homeostasis in obesity. The increased activity of desaturases might be the best explanation. The predominant ω-6 fatty acid is arachidonic acid (20:4 ω 6), which is converted to prostaglandins, leukotrienes and other lipoxygenase or cyclooxygenase products [64] that are regulators of inflammation, atherogenic and prothrombotic effects [64]. The increased levels of AA in the metabolic syndrome group and the further increase over time could be explained with an excessive activity of Δ6 and Δ5 desaturase in subjects with elevated insulin levels leading to an increased production of 20:4 ω -6 from 18:2 ω -6. As insulin activates both enzymes, this situation may occur in subjects with chronic hyperinsulinemia periods [165]. Another ω-6 fatty acid is the essential γ-linolenic acid (18:3 ω 6). This fatty acid is the precursor of arachidonic acid (AA). Basally there were no differences in concentration between the groups, whereas the level of γ-linolenic acid increased over time in the metabolic syndrome subjects as a reaction to the OFTT. In the control group there was no reaction.

Palmitic acid and its transformation products produced by normal or escape pathway

The thesis of chronic and acute changes of the metabolism in metabolic syndrome subjects was supported by the differences between the groups concerning the metabolic pathways of the most common nutritive fatty acid 16:0. One pathway results in lignoceric acid, and the "escape" pathway leads to nervonic acid, which we expected to be elevated in the metabolic syndrome subjects, since the phospholipids containing this fatty acid are associated with an increased mortality [105]. The first step of the escape pathway is the conversion of palmitic acid into palmitoleic acid via Stearoyl-CoA desaturase 1 (SCD-1). Previous publications showed that SCD-1 is increased in cardiovascular diseases, insulin resistance and obesity [106,107]. The 16:1/16:0 ratio which we took as an indicator for the SCD-1 activity was

indeed elevated in the metabolic syndrome group compared to the other groups. Our observation of an elevated basal concentration of palmitoleic acid (16:1 n-7) in plasma of metabolic syndrome subjects is consistent with previous publications [166]. Metabolic syndrome subjects show the highest values, which is a sign of their chronically postprandial state. We also observed an acute reaction to the OFTT in the metabolic syndrome and the risk subjects and no reaction in the control subjects. Zong et al. showed the connection between an undesirable adipokine profile, elevated inflammation markers and elevated erythrocyte palmitoleic acid concentration. The concentration of fatty acids in erythrocytes reflects the concentration of fatty acids in plasma [104]. This is in line with our observations. However, palmitoleic acid (16:1 c-9) not only has unfavorable effects but is also protective against apoptosis of pancreatic cells [167]. As far as FA 18:0 (stearic acid) is concerned, which is known to enhance the development of diabetes [164], our results support recent findings because we observed progressively higher fasting values in the control group, risk group and the metabolic syndrome group. We cannot tell which is the cause and which is effect, but the initial values differ when the groups are compared and moreover, there is a difference in the reaction to the OFTT in all three groups. The control group has its peak after 4 hours and already shows decreased values after 6 hours. The risk group and the metabolic syndrome group have their peaks at 6 hours and decrease thereafter. This again points out the inertia of the metabolism in the metabolic syndrome group. The reason for the stronger increase in the metabolic syndrome group can be found by combining this observation with the observation that the chylomicron level is more elevated after an oral fat tolerance test in the metabolic syndrome group than in healthy subjects. Therefore, the most probable explanation is a higher intake of fat by the intestine in the metabolic syndrome group. Another potential reason is a faster transport from the liver to the plasma due to the observed elevated VLDL levels in the metabolic syndrome subjects. The difference in the fasting values underlines our thesis that the metabolic syndrome subjects are never in an actual fasting state. Looking at the following product in the “normal” pathway, arachidic acid, also called eicosanoic acid (FA 20:0), the metabolic syndrome group again showed elevated levels. This again supports the hypothesis of a chronic adaptation of the metabolism and a chronically postprandial state. The acute changes in metabolic syndrome and risk groups were significant, whereas in control subjects, there was no response to the OFTT. Thus, the risk and the metabolic syndrome groups show, after an initial increase in response to the OFTT, a decrease 6 hours later. The increase can be seen as an overload with a limited capacity to process the fatty acids in other fatty acids or lipid species. Therefore, the FA 20:0 could be a new biomarker for the risk of metabolic

syndrome. Looking at the “escape” pathway, oleic acid and nervonic acid both showed elevated levels in the metabolic syndrome group, whereas lignoceric acid did not show a basal difference between the groups. This result supports the hypothesis that metabolic syndrome subjects use the escape pathway more often than the control subjects. In conclusion, the serum’s fatty acid composition as analyzed by GC-MS shows distinct differences between the metabolic syndrome, the risk and the control subjects. Saturated and unsaturated fatty acid profiles differ basally and over time between the groups. The palmitoleic acid shows basal differences and different responses to the oral fat tolerance test in the three groups. Eicosapentaenoic acid showed different responses over time and the fatty acid 20:0 showed an increase as a reaction to the OFTT in the metabolic syndrome and the risk patients which is not present in control subjects. Concerning oleic and nervonic acid, the oral fat tolerance test provided no further information, but the hypothesis of an altered metabolism with use of the “escape” pathway is supported by the results.

4.2.4 Fasting and postprandial sphingomyelin profile as risk indicator

SPM species associated with unfavorable lipid profile and elevated apoptosis

The distribution of the SPM species in plasma with SPM 16:0 and 24:1, representing the major SPM species, is in agreement with previous studies [168]. Generally, high levels of total SPM are associated with atherosclerosis and CVD [169]. Elevated levels of SPM in individuals with CVD must be partly caused by higher levels of the main species 16:0 and 24:1, since positive mortality associations were described for SPM 16:0 and 24:1 [121]. Even though our results did not show a chronically elevated level in the metabolic syndrome group, they showed a trend towards a higher concentration of SPM 16:0 in the metabolic syndrome group, which was insignificant, most likely due to the small number of cases. Over time, the SPM species 16:0 increased as a response to the OFTT in the metabolic syndrome group, whereas the concentration did not change in the other groups. The fact that the change only occurred in the metabolic syndrome group makes the SPM species carrying palmitic acid a potential prognostic biomarker for the metabolic syndrome. This is in accordance with the result described below, which is that palmitate and stearate belong to the undesirable lipid profile. They have previously been shown to induce apoptosis in cultured cells, and part of cardiomyocyte loss in the genesis or progression of heart failure in humans is mediated by apoptosis [121]. The result shows a significantly higher SPM 18:0 level in the metabolic syndrome group and an elevated level of SPM 18:1. Higher levels of SPM 18:0 and 18:1 have

already been described in mice after high-fat diets and in obese mice [115]. The SPM 22:4 concentration showed decreased levels in the metabolic syndrome group. Since ELOVL2 catalyzes the rate-limiting PUFA elongation steps of ω-6 arachidonyl-CoA (20:4) to docosatetraenoyl-CoA (22:4), and on to tetracosatetraenoyl-CoA (24:4) [121], decreased levels in the metabolic syndrome group support the thesis of an undesirable fatty acid profile with a decreased transformation rate of AA into 22:4 in the plasma. Arachidonic acid itself has not been described in literature as being a part of sphingomyelin species in humans, which is why we did not measure AA-containing SPM species. On the contrary, it has been described that sphingomyelin inhibits the release of AA from membrane phospholipids [170,170].

Sphingomyelin 23:1, the anti-depression species

The SPM 23:1/SPM 16:0 ratio has been described to correlate inversely with depressive symptom scores [171]. Our results showed elevated levels of SPM 23:1 in the control group compared to the other groups. This could support the thesis that SPM 23:1 belongs to the "good" SPM species. The decrease as a response to the OFTT and the ensuing normalization towards the baseline level was only observed in the metabolic syndrome group. This result makes SPM 23:1 a potential biomarker, and the further decrease in the metabolic syndrome group after the OFTT makes it more likely that an undesirable lipid profile is connected with a decrease in SPM 23:1.

Sphingomylin 24:0 as a protective species

In order to confirm this thesis and our results, further tests and examinations will be needed. SPM 24:0 is one of the most protective SPM species [121] and its level was decreased in the metabolic syndrome group. The metabolic syndrome group reacted to the OFTT with an increase in level. This increase could be interpreted as a protective response to an additional fat excess.

4.2.5 Antioxidants: plasmalogen down-regulation in metabolic syndrome subjects

The predominance of plasmalogens carrying DHA and AA in sn-2 position was consistent with the previous literature [112]. Most of the plasmalogen species were initially down-regulated in the metabolic syndrome group compared to the control group, which makes them potential biomarkers. This is in line with findings that plasmalogens, especially as part of

LDL particles, have an antioxidant effect [62]. The results are also consistent with previous studies, as the examination of Colas showed that PE plasmalogens were down-regulated in subjects with T2D [165], whereas in contrast to this Goto`s group showed that plasmalogens were increased in T2D subjects without microangiopathy [172]. The Obesity Society published results showing that uniform increases were observed in plasmalogens and their precursors after short-term overnutrition. They speculated that this was due to plasmalogens as powerful antioxidants and that the increase might be an appropriate response to increased oxidative stress generated by 28-day overnutrition[173].

Protective up-regulation in the metabolic syndrome group due to acute oxidative stress

Their hypothesis could be an explanation for the acute response to the OFTT observed in the metabolic syndrome group since, interestingly, most of the plasmalogen species showed a peak at 6 hours as a reaction to the drink in the metabolic syndrome group, whereas in the control group most species did not respond to the OFTT and those which did had their peak earlier than the metabolic syndrome subjects. Another possible explanation is that this increase in plasmalogen concentration in the metabolic syndrome group is caused by an excess of those fatty acids in the respective plasmalogen which are incorporated into the plasmalogens and are therefore responsible for an increase in the plasmalogen concentration.

In terms of AA containing plasmalogens, fasting values did not differ between the groups, while after ingestion of the OFTT, the metabolic syndrome and the risk groups showed a significant increase in their levels and after 6 hours, the metabolic syndrome subjects showed even higher levels than the control group. These results diverge from previous publications where especially the PE-plasmalogens with sn-20:4 were described as significantly lower in the metabolic syndrome group compared to the control group [174].

Plasmalogens with linoleic acid are down-regulated in metabolic syndrome subjects. Here too, the peak in the metabolic syndrome group occurred 6 hours after ingestion of the drink. In this case, the peak stayed below the levels of the control group. In contrast to previous literature [62] PE (P-16:0/18:0) with stearic acid in sn-2 position, which has been reported to be elevated in oxidative stress [113], is up-regulated in the metabolic syndrome group. Marked differences in fatty acid composition in adipose tissue have previously been published, comparing obese and lean twins. The results included reduced levels of stearic (C18:0) and reduced levels of linoleic (C18:2 ω-6) acid. This work group proposed that the observed lipid

changes were consistent with the proportion of ether lipid [174]. In our results, stearic acid showed increased plasma levels and the PE (P-16:0/18:0) also showed increased plasma levels in the metabolic syndrome group. Changes concerning linoleic acid as acyl residues in sn-2 position reflect in plasma the results this work group found for the fatty acid in adipose tissue. Next, the fatty acid profile was compared to the plasmalogen profile.

4.2.6 Differences in dynamic and baseline plasmalogen levels

This part of the thesis dealing with correlations is exclusively descriptive. No definite conclusion can be drawn from it. A positive correlation between a fatty acid species and a plasmalogen might be a sign of an increased integration of the respective fatty acid into the plasmalogen, the high fatty acid level leading to an increased plasmalogen concentration. A negative correlation, on the other hand, could be a sign of an increased release of the fatty acid from the plasmalogen, the increasing fatty acid causing a decrease of the respective plasmalogen. In view of the results described in the sections above, this is the most probable explanation for the correlation coefficients, which will also be worked with in the following section. In this section we do not care too much about the odd-chain fatty acids, since these odd-numbered fatty acids are only biomarkers for the intake of dairy products [175]. The fatty acid 13:0 was used as an internal standard, but slight variations of the levels among the individuals existed because this fatty acid also exists in traces in human serum.

The fact that the medium-chain fatty acids positively correlated only with the plasmalogens carrying 18:0 in sn-2 position in the control and the risk groups but with all of the plasmalogens species carrying a saturated acyl residue in sn-2 position in the metabolic syndrome group subjects might indicate that the medium-chain fatty acids are primarily elongated and integrated in the 18:0 species, and after a certain level could be achieved in the risk and metabolic syndrome groups, the other plasmalogens species carrying an saturated acyl residue were filled up. In biosynthetic pathways, plasmalogens serve as a reservoir for PUFAs, since they preferentially contain arachidonic (20:4), docosapentaenoic (22:5 n-6) and docosahexanoeic (DHA, 22:6) acid in the sn-2 position [176]. Eicosanoid biosynthesis begins with the hydrolysis of fatty acids from the sn-2 position of neutral membrane glycerophospholipids to produce free fatty acids and lysophospholipids upon receptor-mediated events [177]. Arachidonic acid is actively incorporated into the membrane phospholipids and selectively targeted to ethanolamine plasmalogens [174]. Low levels of PUFAs correlate with decreased plasmalogen levels [178]. Low levels of polyunsaturated fatty acids are a sign of low levels of PUFAs containing plasmalogens which cannot be built,

and the degradation of those plasmalogens carrying PUFAs is elevated. An elevated degradation of PUFAs containing plasmalogens is a sign of oxidative stress [178]. In literature, especially the interrelations described above, between plasmalogens containing arachidonic (20:4), docosapentaenoic (22:5 ω-6) and docosahexanoeic (DHA, 22:6) acid and PUFA serum levels, are described. EPA, which showed increased levels in the plasma over time in the metabolic syndrome group, could indicate a release by the plasmalogens. The fact that arachidonic acid (20:4 ω-6) correlated negatively with the plasmalogens carrying linolenic acid (18:2 ω-6), the precursor of arachidonic acid (AA) [179], in the control group but not in the metabolic syndrome group could be an indication that the proinflammatory arachidonic acid is produced additively by another way in the metabolic syndrome group than in healthy subjects. Since sn-2 (R2) is esterified predominantly with ω-6- or ω-3-derived polyunsaturated fatty acids [180,181], it was not surprising that the highest prevalence of significant correlations with PUFAs occurred between the plasmalogens carrying arachidonic acid in sn-2 position. If one PUFA is up-regulated in serum, it can serve plasmalogen synthesis and AA in plasma can derive from the appropriate plasmalogen species. It is noticeable that the arachidonic acid 20:4 (n-6) correlated with the named plasmalogen species in the control and risk groups, e.g. species carrying 22:4 in sn-2 position, whereas in the metabolic syndrome group it only correlated positively with plasmalogens containing 20:4. This might indicate that in the metabolic syndrome group, arachidonic is most often integrated as arachidonic acid itself, but in the other groups its less harmful desaturation product docosatetraenoic acid 22:4 is integrated. The deduced fact that it can also be converted into 22:4 and stored in plasmalogens in the control and in the risk groups matches the results of the comparison of plasmalogen species between the three groups described above, in which PE (P-18:1/22:4) showed a significant difference in baseline levels between the groups . Linoleic acid (18:2 n-6) positively correlated with PE (P-18:1/20:4) in the risk and metabolic syndrome groups, but not in the control group. Thus, the level of linoleic acid may have been elevated in the metabolic syndrome and risk groups, but the concentration in plasma was not significantly different between the groups, because its elongation and desaturation product arachidonic acid is generated and integrated in the plasmalogen. The case of all plasmalogens containing 20:4 in sn-2 position correlating with FA 24:1 (n-9) only occurred in the metabolic syndrome group. This supports the fact that the metabolic syndrome subjects have an undesirable fatty acid profile with elevated nervonic acid which leads to death and that an up-regulation of plasmalogens containing arachidonic acid takes place. α-linolenic 18:3 (ω-3) positively correlated with the plasmalogens carrying DHA in sn-2

position in the risk and metabolic syndrome groups, contrary to γ-linolenic acid which did not correlate with any of the plasmalogen species in these groups and showed a discrete but not significant negative correlation value in the metabolic syndrome group when correlated to the named plasmalogen species. This is not astonishing, since linoleic 18:2 (ω-3) and α-linolenic 18:3 (ω-3) are precursors of DHA, and γ-linolenic 18:3 (ω-6) is a precursor of the proinflammatory arachidonic acid 20:4 (ω-6). But the described correlation phenomena were not observed in the control group. This might be due to the fact that in the control group, the DHA reservoir in plasmalogens is full, it is not necessary to adapt plasmalogen levels containing DHA acyl residues to the concentration of its precursors, for example by increasing DHA synthesis and the incorporation of DHA into plasmalogens. The results described above showed that the PE (P-18:0/22:5) baseline level was reduced in the metabolic syndrome group; this would support the thesis, that the DHA containing plasmalogen levels could be sustained by the processing of their precursors. The reduced level of DPA as a precursor of DHA could not be compensated anymore, whereas DHA production could be maintained due to an increased production from the precursors.

4.2.7 Proapoptotic and protective ceramides and risk for metabolic syndrome

Another potential biomarker in fasting and postprandial state for the risk of metabolic syndrome could be the ceramide ratio 24:0/24:1. The ceramide 24:0 is known to have antiapoptotic properties [182], but ceramide 24:1 is responsible for cell death [183]. We calculated the ratio of Cer 24:0/24:1 and found a higher ratio of Cer 24:0/24:1 in the control subjects. This result shows a higher rate of the "survival" or protective ceramide 24:0 and a lower rate of the "death" ceramide 24.1 in healthy subjects. Majumadar et al. found no association between metabolic syndrome and elevation of the ceramide 24:0 in their study with children, but they found an elevation of this ceramide species in association with insulin resistance and inflammation [184]. Another study in which total ceramide levels showed a tendency toward increased levels in sepsis patients described an inverse behavior of the two main species, Cer 24:1- and Cer 24:0 [108].

4.2.8 Low LPC species, increased mortality and sepsis

It has been published previously that LPC levels increase while the plasmalogen levels decrease in metabolic syndrome subjects. High LPC and low ether phospholipids result in oxidative stress. LPC produced by the impact of the proinflammatory phospholipase A_2 on

phosphatidylcholine (PC) promotes inflammatory effects, including an increased endothelial expression of adhesion molecules and an activation of the growth factors monocyte chemotaxis and macrophages [108]. In contrast, this study observed lower LPA and LPC concentrations in the metabolic syndrome group compared to the other groups. A lower level of LPC correlating with unfavorable metabolic aspects resulting in an increased mortality risk is supported by a study which showed that the total LPC level was markedly decreased in sepsis patients [108]. In addition, a study finding a decreased LPC concentration in obese subjects has been published previously [65]. Another fact which supports our results is that lecithin-cholesterol acyltransferase (LCAT) activity, a major source of LPC in circulation, has been shown to be decreased in obesity. This potentially leads to a decrease in circulating LPC [169]. Another work group found higher concentrations of LPC 14:0 and LPC 18:0 in overweight/obese men, while LPC 18:1 was found reduced [115]. LPC 18:2 and sphingomyelin 16:1 in serum were found inversely related to type 2 diabetes risk [115]. Our results also showed that the reduced levels of LPC 18:1, 18:2, 18:3, 20:3, 20:4, 20:5 and 22:5 were responsible for the reduced LPC levels in the metabolic syndrome group compared to the other groups. A study using mice which were fed a high fat diet for 12 weeks and again for 14 weeks showed induced levels of LPC 17:0, 18:0 and 18:3, while most of the other species decreased in the fat-fed mice [115].

Acute increase of LPC species reflects chronic increase

This is applicable to our results, which showed significantly induced LPC 18:0 in the metabolic syndrome group as early as 4 hours after the OFTT; LPC 18:3 increased in all three groups. Even though we did not observe an elevation of the baseline level in the metabolic syndrome group, we found induced levels of the LPC species 18:0 and 18:3 as a response to the OFTT in metabolic syndrome subjects. It can thus be assumed that an additional fat-load leads to a short-term/acute elevation of LPC 18:0 in subjects which already have an undesirable metabolic profile. These results, which show an increase of LPC 18:0 as a response to the OFTT 4 hours after a high fat load in subjects with an already chronically elevated fatty acid profile, support the study with mice mentioned above. In that investigation LPC 18:0 increased after a certain period of a high-fat diet resulting in an undesirable metabolic profile.

The reduced levels of LPC 18:1 in obese subjects that have already been described [115] were also reflected in our study. According to those examinations, S 20 showed down-regulated baseline levels in the metabolic syndrome group, while the LPC 18:1 level was induced in all

three groups as a response to the OFTT over time. This makes LPC 18:1 an appropriate indicator for long-term changes in lipid profile, applicable to the metabolic syndrome.

LPC has also been identified as being a ligand for the immunoregulatory receptor G2A, which is predominantly expressed in immature T-and B-cells [108], where G2A is coupled to inhibit Gi proteins, while G2A in PMN-innate immunity cells is coupled to activate Gα and Gq. Based on previous data, LPC may force a shift of the immunomodulatory pathways resulting in an activation of the unspecific immune system (PMN) and the suppression of the specific system (T- and B-cells). Other authors proposed that the decreased LPC concentration might reflect its enhanced conversion to lysophosphatidic acid (LPA) by the activity of a plasmatic lysophospholipase D [108].

4.2.9 Lower immunomodulatory lysophosphatidic acid in metabolic syndrome subjects

LPA binds itself to different G-protein receptors from the edg-receptor family (edg-2, edg-4, edg-7) and is known to induce a multitude of cellular responses, including LPA-driven effects on immune cells such as the promotion of T-cell and macrophage survival, and increased endothelial adhesion molecule expression [108]. Moreover, LPA-mediated activation of edg-2 on T-cells was shown to stimulate interleukin-2 production and inhibit cell migration, whereas binding itself to edg-4 had the opposite effect [108]. In the case of our results, where LPA also showed decreased levels in the metabolic syndrome group, the shift provides no sufficient explanation. The inverse behavior of the LPA levels between the different groups is in contrast to another publication, which states that high LPA concentrations positively correlate with obesity [67].

The result suggests an integration of a fatty acid and its desaturation products in LPA species after an increase of the fatty acid concentration as a response to the OFTT. Those LPA species are probably converted into other phospho- or lysophospholipids. It may be speculated that the protective effect is based on withdrawing pro-inflammatory fatty acids by integration and LPA species by conversion from their signaling pathways and the resulting formation of LPC species which appeared to be associated with a protective effect. In the metabolic syndrome group, the conversion of LPA into LPC species LPC 18:2 and 20:4, associated with positive effects, might not be sufficient. In those cases, the oral fat tolerance test was able to provide further information about the metabolic state. Additional tests will be needed to explore the usefulness of this postprandial dynamic as a biomarker.

4.2.10 Correlations between PC and LPC species

Baseline differences between groups due to chain-length of PC species

Regarding the PC species it is remarkable that when comparing the baseline levels of the control and metabolic syndrome groups, those with short-chain acyl residue showed higher baseline levels in the control group while those containing more C atoms showed higher values in the metabolic syndrome group. It has been published previously that four LPCAT subtypes responsible for LPC to PC conversion have been identified and each LPCAT has different substrate specificities [185]. This thesis concentrated on the behavior of the PC species in the groups over time, which was especially analyzed by considering the formation of Lyso PCs. It is known that enzymatic hydrolysis of one fatty acid (FA) mainly by phospholipase A2 activity leads to Lyso PC (LPC) species formation, which act as highly abundant signaling molecules through specific G-protein coupled receptors [9]. A recent study revealed associations between phospholipase A2, LPC, lysophosphatidic acid and proinflammatory cytokine levels [121]. In the control group, certain PC species were downregulated while the fatty acid species and LPC species were upregulated. This relation is less present in the metabolic syndrome group.

Refilling of protective PC species

The thesis of a conversion of PC species into the corresponding fatty acid and LPC species, and especially differences in the conversion behavior in the metabolic syndrome subjects (as adaption mechanism to chronic fat overload), is supported by the increase of PC 32:2 in the metabolic syndrome and in the risk group and a simultaneous increase of the fatty acid 14:0 and LPC 18:2 and by the increase of LPC 18:3, while PC 32:3 decreased and the fatty acid 14:0 increased as a response to the OFTT after 8 hours in the control group. In the metabolic syndrome group, the increase of fatty acids does not co-occur with a decrease in PC species at all. The fact that PC 32:2 is still increased after the conversion indicates that PC 32:2 may be refilled by another mechanism such as the desaturation of PC 32:0. The delta-9-desaturase (D9D) stearoyl-CoA desaturase (SCD/FADS5/D9D) shows a specific affinity to the two main dietary saturated FAs palmitate and stearate, converting them into the corresponding monounsaturated FAs palmitoleate (16:1n-7) and oleate (18:1n-9). This indicates a protective role of SCD in PC 32:1 and PC 32:2, which contain SCD desaturation products. Compared to PC 32:0, the presence of the two PC species indicates a lower mortality risk, with PC 32:2 being slightly but significantly protective [121]. Another interesting observation in the risk

group was that PC 36:5, which can contain 20:4 and so far is supposed to be protective against CVD [121], increased at 6 hours as a response to the OFTT, while FA 20:3 was also increased but the plasma concentration of 20:4 did not change, indicating a withdrawal of FA 20:4 by the formation of PC 36:5. The saturated and monounsaturated PC species identified are positively associated with mortality, with PC 32:0 revealing the strongest positive association. PC 32:0 is most likely composed of two palmitates (16:0) at sn-1 and sn-2 position; alternatively myristate (14:0) and stearate (18:0) could also form PC 32:0 [121].

Loss of SCD's protective function in the metabolic syndrome group

PC 32:2 is a protective species associated with reduced mortality [121]. It was induced as response to the OFTT in the control group. Palmitoleic acid 16:1 (n-7) was also increased. This indicates an activity of SCD, which catalyzes the conversion of palmitic acid (16:0) into palmitoleic acid, in this case a protective procedure. After 6 hours, 32:1 – another PC associated with a reduced mortality compared to PC 32:0 [121] – was induced. In the risk and metabolic syndrome groups, PC 32:2 also showed induced levels as a response to the OFTT, but without an increase in PC 32:1 in the metabolic syndrome group. This indicates a loss of the protective function in the metabolic syndrome group and needs further research.

PC 32:0 strongly associated with morality

The PC 32:0 level, having the strongest association with mortality [121], did not change remarkably in any of the groups over time, even though the fatty acid species that this PC species contains were upregulated in all the groups after the OFTT. But it is one of the short-chain PC species that shows the rare phenomenon of a reduced baseline level in the control group compared to the metabolic syndrome subjects.

Postprandial release of DPA in the control group

The fact that the level of PC 42:5 was reduced 8 hours after the fat load in the control group indicates that the docosapentaenoic acid (22:5) containing PCs releases the protective fatty acid.

Elevated baseline levels, reduced possibility for further elevation

The PC species 40:2, 26:0 and 40:5 have been described earlier to be induced in obesity [115]. For those PC species, we only found elevated baseline levels in the metabolic syndrome group. The control group, showed an elevation of 40:2 over time, which could not be

observed in the metabolic syndrome group. In the risk group even a reduction was seen after the OFTT application. This could be interpreted as being due to basally elevated levels in the metabolic syndrome and risk groups, which leaves less room for an elevation as a reaction to the OFTT.

Loss of FADS1/D5D-activity

Another factor to consider were the enzymatic pathways. FADS1 catalyses delta-5 desaturation (D5D), converting eicosatrienoyl-CoA (20:3) to arachidonyl-CoA (20:4). These FAs can for example be found in the PC species 36:3 and PC 36:4, and the ratio between PC 36:3 and 36:4 is regarded as a good predictor of FADS1/D5D activity [121]. The ratio of PC 36:3 and 36:4 as good predictor of FADS1/D5D-activity, which reflects the conversion of eisosatrienoyl-CoA (20:3) into arachidonic acid (20:4) [121], unfortunately showed no remarkable differences over time. But on a basal level, the metabolic syndrome subjects showed a lower ratio, indicating less activity of the enzyme. Delta-6 desaturase (D6D)/FADS2 may be involved, generating octadecadienate (18:2n-9) from oleate. This effect is less pronounced for PC 36:2 and PC 38:2, although a similar tendency is visible in 10 PC species containing long-chain PUFAs, which were associated with a protective effect in previous analysis [121]. In the risk and the metabolic syndrome groups, PC 38:2 showed reduced values over time, which could indicate a reduced activity of this enzyme.

Oppositional effects in the modulation of the inflammation systems

PC species containing long-chain saturated and monounsaturated n-9 FAs positively associate with mortality, whereas long-chain PUFAs appeared to be associated with a protective effect [121]. It may be speculated that the protective effect is based either on the withdrawal of pro-inflammatory eicosanoids of the n-6 series from their signaling pathways or on the provision of anti-inflammatory eicosanoids of the n-3 series.

4.2.11 Inflammatory markers and chronic inflammation in the metabolic syndrome

With this work we not only succeeded in confirming chronic long-term inflammation in metabolic syndrome subjects or the tests conducted e.g. by Lemieux et al., which showed that fasting C-reactive protein (CRP) correlates with visceral adipose tissue mass [186], but we also showed that in terms of the inflammation state, there is a change in the postprandial behavior in response to a high fat load in metabolic syndrome subjects. It is known that CRP

is not associated with fasting dyslipidemia but with the metabolic syndrome and CHD risk [186]. In addition, we found a significant decrease in CRP in the metabolic syndrome group two hours after the oral fat load. Such an examination has not been made until now. This supports our thesis of chronic and acute changes in the body composition of metabolic syndrome subjects. CRP could therefore serve as a new biomarker for metabolic syndrome by applying the oral fat tolerance test. Another work group looked for CRP concentrations after an oral fat challenge in a healthy collective [187]. They found an increase in CRP concentration 6 hours after the fat load. This slight increase was also observed in our study, although it did not reach statistical significance (p=0.291). Since their OFTT did not differ much from ours, with the fat content being only 2% higher, but included a much larger study population (>100 participants), we can assume that the observed change in our subjects might have been significant if our groups had been larger. Another inflammation parameter, suPAR, is known to be a predictor for CVD [124], but the suPAR levels in the different groups have not been compared yet. The reaction to an oral fat load has not been tested yet either. Since basal suPAR levels are significantly higher in the metabolic syndrome subjects, the suPAR level under fasting conditions could serve as a biomarker for the metabolic syndrome.

4.2.12 Functional changes of the adipose tissue

Omentin

Subsequently, we dedicated our attention to the adipose tissue. Omentin – a cytokine which is specifically expressed by the visceral fat tissue – was analyzed. It has an important influence on paracrine and autocrine ways of fat distribution and obesity-associated diseases. It also has endocrine effects on insulin enhancement. Omentin attenuates the CRP concentration and inflammation pathways [129]. It is already known that the omentin concentration is decreased in metabolic syndrome subjects [129]. We were able to confirm chronically decreased levels of omentin concentration in metabolic syndrome subjects compared to control subjects. Since the response to the OFTT was the same in each group, the OFTT unfortunately provided no further information.

Leptin

However, we did find a prognostic advantage of administering the oral fat load for another cytokine, leptin, which is also mainly expressed by adipocytes [55]. Our results confirm previous publications, as the basal leptin concentration was significantly higher in the risk and

the metabolic syndrome subjects compared to the control subjects. Moreover, our results showed that there were acute leptin responses during the 8 hours following an oral fat tolerance test in the metabolic syndrome and the risk subjects. This is a deviation from the results of the group of Guerci et al. [188], who observed the reaction of control and obese subjects to an oral fat load and then compared the two groups' reactions. Their results indicate that there is no acute response, neither in obese nor in slim subjects. A possible explanation for the differing results is that our stratification criteria for the groups differed from theirs. Moreover, we did not only compare subjects with different body fat compositions, but also subjects with metabolic syndrome, a risk and a control group. It is known that leptin is regulated by the adipose tissue mass and by food intake [188]. But Guerci et al. suggested in their review that there is a lack of postprandial regulation by fat intake [188]. Our results prove otherwise. The fact that our OFTT consisted of 66% fat and their drink consisted of 85% fat may be a disadvantage of our study. But since over 30% of our drink were carbohydrate and only 3% proteins, the protein concentration can be ignored. Our study population was larger than that of Guerci et al., who only tested 32 persons and included men and women. The reason for the striking decrease in leptin concentrations 4 hours after the fat load in the metabolic syndrome group is not clear. But since the decrease took place at 12 o'clock and not at 10 o'clock, it cannot be explained with a previously published paper, which reports a nadir of the leptin concentration at 10 o'clock due to the circadian rhythm [188]. Our results for the chronically different basal concentrations of leptin in the three groups are in good agreement with previous reports [188]. Thus, in summary our results provide evidence that fat intake is only linked to an acute change in postprandial leptin in the metabolic syndrome and the risk subjects, whereas in the control group the leptin concentration is not acutely influenced by the oral fat tolerance test.

Adiponectin

Already in 1996, Spiegelman et al. demonstrated that low adiponectin levels in plasma correlate negatively with obesity in rodents [189]. We also found decreased levels in the metabolic syndrome group compared to the other groups. In 2005, Kadowaki's group stated the role of adiponectin in insulin resistance and as an antiatherosclerotic hormone [54]. Adiponectin has protective effects on the kidney, heart and pancreas due to its antiapoptotic effects [54]. Fruebis et al. found out that adiponectin negatively correlates with postprandial fatty acid levels in the blood [190]. A further global effect of adiponectin is a stimulation of the lipid catabolism. It is well-known today that adiponectin increases ß-oxidation [191]. The

adiponectin dynamic after an oral fat challenge has not yet been evaluated. Annuzi et al. already compared the reaction of 10 diabetics with the reaction of non-diabetics to a normal meal. Adiponectin in diabetics decreased 6 hours after the meal, but there were no significant changes in the other group [192]. We looked at the human serum adiponectin levels in vivo before and during OFTT and compared the control, risk and metabolic syndrome subjects' response. The dynamics of adiponectin have not yet been measured this way. Our results showed that the control group had the highest, the risk group had lower and the metabolic syndrome group had the lowest adiponectin levels. This is in agreement with many recent publications. This obvious trend in the three groups, was not significant at time zero. With a p-value of 0.150, the observation of the basal differences in the three groups could not be confirmed by the first statistic calculation. But since the groups were very small [n=14 (control, 0h) and n=31 (metabolic syndrome, 0h)], it was more representative to include all points in time to show that the mean adiponectin concentration was significantly different in the three groups. As expected, there was a significant difference between the adiponectin levels in the groups. The adiponectin level of the control group was significantly higher than in the other groups. We observed a significant difference in a reaction to the OFTT. There was a change in the metabolism of the metabolic syndrome subjects, which again suggests the thesis of a retarded and reduced reaction to the OFTT. In the control and the risk groups, the decrease of the adiponectin concentration as a response to OFTT was striking and significant. Summarizing, there was a chronic change in metabolism, as well as a change in the acute response to OFTT. The lower basal levels in the metabolic syndrome groups and the absence of a reaction again confirms the hypothesis that metabolic syndrome subjects are in a chronically fed state, which was reflected by their basal levels, and that there was no acute response to the OFTT because the metabolism function shifts towards metabolic resistance. In Figure 58, a minimal reaction on the OFTT is visible, but the change in time is not significant. With a 95% probability, there is no reaction to the oral fat tolerance test in the metabolic syndrome group. The visible and similar trend in all groups, with a peak at 4 hours after the oral fat load, is particularly interesting. The same development was previously shown with women fed a carbohydrate-dominated meal [193]. Since the half-time of adiponectin is between 65 minutes in lean and 75 minutes in obese subjects [194], it is either possible that after this time, adiponectin secretion increases again but does not reach the basal secretion rate and adiponectin is cleared again rapidly, or that secretion is cut back again. Our results showed that postprandial adiponectin levels are more informative with regard to the metabolic state than fasting adiponectin levels.

Leptin-adiopnectin ratio

We also looked at the leptin-adiponectin ratio which is a better predicting factor for CVD than leptin or adiponectin alone [133]. The metabolic syndrome and the risk groups showed a significant response to the OFTT, with an elevated leptin-adiponectin ratio. This could be interpreted as a shift toward the non-favorable cytokine composition as a response to the OFTT. The control subjects did not show any significant change in the leptin-adiponectin ratio. Here, the OFTT could be used to predict the risk of the metabolic syndrome.

Ghrelin

For another adipokine, ghrelin, we also found interesting results which will require further exploration, since our number of valid cases was too small. “Ghrelin is a growth hormone-releasing peptide that has been shown to improve cachexia in heart failure and cancer and to ameliorate the hemodynamic and metabolic disturbances in septic shock.” [195] The fact that cytokine-induced inflammation is critical in these pathological states, and that the growth hormone secretagogue receptor has been identified in blood vessels, led to a study by Li which confirms the thesis that ghrelin inhibits proinflammatory responses in human endothelial cells [195]. Ghrelin is not only secreted by the adipose tissue but is mainly synthesized and secreted in the stomach and the intestine [196]. It reaches its maximum plasma levels before meals in order to stimulate food intake [196]. We examined des-acyl ghrelin, which accounts for 90% of the circulating ghrelin [197] and which plays a role as an antiapoptotic, antilipolytic hormone improving medium-chain FA uptake. In this way, it acts against lipotoxicity and inflammatory responses [198]. Its role in hunger has been described differently by different authors, though acylated ghrelin has an orexigenic effect [197]. Morever, des-acyl ghrelin improves glucose tolerance. Roux et al. have already shown that fasting ghrelin is lower in obese subjects than in control subjects, which confirms our thesis that obese or metabolic syndrome subjects are in a postprandial state. Ghrelin increases hunger [199]. Its secretion is suppressed depending on the caloric intake, and also depends on the food composition [199]. In the decrease after a normal meal, the nadir generally lies between 2-4 hours after the meal [200]. We observed fasting ghrelin levels and how ghrelin levels developed during OFTT after four hours. Erdmann observed a decrease of ghrelin levels after an OFTT, but an increase after protein load [201]. At four hours, we found no significant change in ghrelin levels in control subjects compared to the concentration at time zero. In metabolic subjects, the ghrelin levels were lower four hours after the drink, which shows that ghrelin levels were still suppressed. The first interpretation of Figure 60 A would

be that the nadir of ghrelin in control subjects lies before four hours and basal ghrelin levels are reached again four hours after the OFTT, whereas in metabolic syndrome subjects the thesis of the inert metabolism can be supported since the basal levels were not reached yet at that point. Since the number of cases in which both points in time were measured were very few, and since the standard error is very large due to the fact that many values lie beyond the detection value, graphs B-D depict each single case, including the cases lying beyond the detection limit. For this purpose the values beyond detection limit were set on the detection limit: 0.2 pg/ml. The graphs show decreased ghrelin levels at four hours compared to the basal value in most of the cases in each group. The decrease is not as large as in the metabolic syndrome subjects, where most of the values were below detection value. This might be either due to the fact that the ghrelin concentration does not decrease as much in the control subjects as it does in the metabolic syndrome subjects, or the fact that – as mentioned above – the ghrelin values were not on the way to return to their basal values yet in the metabolic syndrome group, whereas the control group shows a return to the values. Further tests on the ghrelin concentration at two hours should be conducted in order to clarify this. This could reveal whether the hypothesis of the inertia of the metabolism in metabolic syndrome subjects can be confirmed by their ghrelin concentration. To evaluate whether the OFTT can be useful in the case of ghrelin, further studies should feature a larger population and a longer time span.

4.2.13 Changes in the renal system and FGF-23 in the metabolic syndrome

Next, we took a look at the kidney. In order to examine kidney values, two parameters were explored: creatinine and FGF23**.** Both parameters showed significantly higher levels in the metabolic syndrome subjects than in control subjects. Our results were consistent with the recently published results by Mirza et al. [202]. This work group found an association between elevated FGF23 levels and lowered HDL, increased TAG and higher BMI [150]. But in contrast to them, who stated "we did not find any significant evidence for the relationship between FGF23 levels and the number of metabolic syndrome criteria met", we did find higher levels in the metabolic syndrome group than in the risk group – which fulfilled only one or two criteria of the metabolic syndrome criteria – and higher levels in the risk than in the control group. Recent publications showed that lower adiponectin and elevated leptin levels are associated with elevated FGF23 levels [202]. Leptin directly stimulates FGF23 expression in the bone [202]. All those findings were not surprising, as the other fibroblast

growth factors – which have structural features in common with FGF23 – also play a role in the lipid and glucose metabolism [202]. Mirza et al. found a link between FGF23 and CVD [202]. FGF23 levels significantly increased in the metabolic syndrome subjects, but they did not change in the control subjects. Since the FGF23 level is associated with kidney function [203] and diabetes [204], it is useful to look at the FGF23 levels in subjects without kidney failure and without diabetes. Normal kidney function is defined by a creatinine level lower than or equal to 1.1. The presence of chronically elevated FGF23 levels in the metabolic syndrome subjects was independent of kidney function. This renders FGF23 useful as a new biomarker for the metabolic syndrome. Regarding the behavior of FGF23 values of non-diabetic subjects with normal kidney function over time, we found that the different behavior of the groups supported the thesis of the inertia of the physiological functions in metabolic syndrome subjects. Further analyses will need to be conducted using larger populations.

5 Summary

In this study, we examined the influence of an oral fat tolerance test (OFTT) on the plasma levels of lipids, inflammatory biomarkers in human serum and biomarkers of the kidney, the bone, adipose tissue and blood compartment in order to test whether metabolic syndrome patients exhibited acute or chronic alterations in their metabolism. To this end, 67 volunteers were stratified into three groups according to the IDF criteria metabolic syndrome, diabesity risk and control. Blood samples were taken following a strict 12-hour fasting period at time points 0, 2, 4, 6 and 8 hours after consuming a high-calorie shake. The shake consisted of 250 ml cream, 100 ml fruit juice and 60 g sugar, resulting in 3% protein, 66% fat and 32% carbohydrate, with a total energy content of 1016 kcal. Samples were then analyzed for their lipidomic profile, inflammatory markers as well as cellular parameters and cytokines. In fact, metabolic syndrome subjects showed an inert metabolism, but also a loss of protective functions, such as the decrease of DPA levels containing PC 42:5 due to the release of protective docosapentaenoic acid 22:5 as a response to high fat load, which could be observed in the control group. On the other hand, other protection mechanism seemed to be recruited. This study supports the thesis, also stated in a previous publication involving sepsis patients with reduced LPC levels [108], that reduced LPC levels are part of an unfavorable lipid profile, as LPC levels (mostly LPC 18:1 and 18:2) were reduced in the metabolic syndrome subjects in our tests. This may also have been a protective mechanism. Another one may be the withdrawal of pro-inflammatory fatty acids by the conversion of certain LPA into LPC species, which our results indicate. They also suggest that this protective mechanism is not sufficient to reach a more favorable lipid profile. The unfavorable lipid profile of the metabolic syndrome subjects included lower levels of anti-oxidative plasmalogens, protective SPM species 24:0 [121] and the LDL/apoB-100 ratio as a sign of smaller and more atherogenic LDL particles. It also showed a decreased transformation rate of AA into 22:4, which was reflected by a decreased level of SPMs carrying the elongation product of 22:4 and 24, an increase in basally elevated FA 18:0, FA 20:0 and SPM species containing acyl residues 18:0 and 18:1. Obesity-related PC 26:0, 40:2 and 40:5 [115] and mortality-associated [121] PC 32:0, PCs 38:4 and 40:6, which have been described as being raised in fat-fed mice and as positively correlating with fasting glucose [115], were basally increased in the metabolic syndrome group. In terms of ceramides, the results showed a higher rate of “survival” or protective ceramide 24:0 and a lower rate of “death” ceramide 24:1 in healthy subjects compared to the others. The SPM containing 16:0, which also belongs to an

unfavorable lipid profile [121], increased over time in the metabolic syndrome group as a response to the OFTT. The thesis that metabolic syndrome patients are not able to reach a proper fasting state anymore, but stay in a constant postprandial condition which is resistant to fat load, was supported by markers showing basally increased levels in the metabolic syndrome group, but only a minor reaction or increase in serum in the control group, such as apoB-48 and PC 40:2. The simultaneous increase in PC 32:2 and 16:1 (n-7) as a response to the OFTT in the control group supported this thesis and, moreover, indicated a difference in SCD enzyme activity between the groups. Another conversion mechanism by which LPC is converted into the corresponding PC species by different LPCAT subtypes [185] indicates different degrees of predominance and efficacy in the different groups. The results indicate different concentrations of the various LPCAT subtypes responsible for LPC-to-PC conversion [185] in the different groups. As an example, LPC 18:3 and fatty acid 14:0 increased, while PC 32:3 decreased only in the control group as a response to the OFTT. Moreover, the results support the thesis that metabolic syndrome subjects are in a chronic state of inflammation, reflected by elevated leukocyte, SuPar and CRP levels, accompanied by an elevated level of proinflammatory w6 fatty acids in plasma. A significant decrease of the CRP level could only be observed in the metabolic syndrome group as a response to the drink. Correspondingly, the correlation of plasmalogens with the fatty acids indicates that the elevated fatty-acid concentration in metabolic syndrome patients causes an increased integration or transformation of the fatty acid into the plasmalogens. Omentin, adiponectin and ghrelin were analyzed as marker cytokines for the regulation of adipose tissue metabolism. Basal levels of these adipokines were significantly lower in the metabolic syndrome group than in the control group, indicating chronic long-term adaptations of the adipose tissue in metabolic syndrome subjects. We found delayed and – in some cases – diminished responses of the groups to the OFTT, which indicates an acute change in the metabolic response. Serum levels of creatinine and FGF23 were significantly higher in metabolic syndrome than in control subjects and further increased over time. Kidney function-associated FGF23 was separately analyzed in subjects without impaired kidney function or diabetes. Remarkably, chronically elevated FGF23 levels in metabolic syndrome subjects as well as their different acute reactions were independent of kidney function. In conclusion, the results of our study shows that an OFTT represents an appropriate means of exerting metabolic stress on subjects in order to increase the sensitivity in laboratory measurements related to pathologies of the lipid metabolism.

6 Zusammenfassung

Der orale Fetttoleranztest zur Bestimmung der Nüchtern- und Postprandialwerte im Serum (2,4,6,8 Stunden nach Aufnahme eines oralen Fettshakes; 1016 kcal und 66% Fettanteil) hinsichtlich spezifischer ausgewählter Lipidspezies, Signalpeptide und Hormone, eignet sich besser als herkömmliche Methoden zur frühzeitigen Risikostratifizierung der Entwicklung eines metabolischen Syndroms. Dies geht aus unserer Studie mit 67 Probanden, die in eine Kontroll-, Risiko- und Metabolische Syndrom Gruppe (nach IDF Kriterien für Metabolisches Syndrome) eingeteilt wurden. Die sich gut zur Risikostratifzierung eignenden Unterschiede zwischen metabolischen Syndrom-, Risiko- und Kontrollgruppe, basieren auf einer Anpassung des Stoffwechsels des metabolischen Syndroms an die chronische Fettbelastung. Patienten, die von einem metabolischen Syndrom betroffen sind, weisen einen trägeren Stoffwechsel auf im Vergleich zur Kontrollgruppe, mit zum Teil verlangsamtem Abbau der Metaboliten sowie einen trägeren Anstieg von Marker Peptiden und Lipiden bei chronisch erhöhten Nüchtern-Werten. Bei Patienten mit metabolischem Syndrom fehlen zum Teil protektive Mechanismen, die bei Gesunden als Reaktion auf erhöhte Fettbelastung einsetzen, wie z.B. die Abnahme der DPA enthaltenden Spezies PC 42:5, als Reaktion auf den OFTT, welche bei der Kontrollgruppe beobachtet wurde und zur Freisetzung der protektiven Docosapentaensäure (22:5) führt. Dagegen setzen wiederum neue Protektionsmechanismen ein. Auffallend waren chronisch erniedrigte Gesamt-LPC-Werte, vor allem bedingt durch die verringerte Konzentration der Hauptspezies LPC 18:1 und 18:2 bei Patienten mit metabolischem Syndrom. Dieses Ergebnis passt zu einer Studie [106], in der diese bei Sepsispatienten erniedrigt und somit als Teil eines unvorteilhaften Lipidprofil zu werten sind. Die Reduktion könnte auch protektiv bei Risiko Patienten gewertet werden unter der Annahme, dass LPC positive Effekte haben. Ein weiterer protektiver Mechanismus ist die Entfernung proinflammatorischer Fettsäuren aus dem Plasma aller Probanden über eine Konversion von LPA und einer spezifischen Fettsäure zu LPC, für die unsere Ergebnisse Hinweise liefern. Die Konversion scheint bei Patienten mit metabolischem Syndrom nicht auszureichen, um ein günstigeres Lipidprofil zu schaffen. Zum ungünstigen Lipidprofil der Patienten mit metabolischem Syndrom gehören ein geringes LDL/apoB-100-Verhältnis als Zeichen kleinerer, atherogener LDL-Teilchen, erhöhte Werte der SPM 18:0, 18:1, PC 26:0 und wie vorbeschrieben der langkettigen PC Spezies insbesondere des PC 40:2 und PC 40:5 als Marker für Adipositas [113] sowie des PC 32:2, des mortaliätsassozierten PC 32:0 sowie der PCs 38:4 und 40:6, die in Mäusen positiv mit dem nüchtern Glucose Wert korrelieren

[113], eine niedrigere Rate an “survival” Ceramid 24:0 und eine geringere Konzentration von “death” Ceramid 24:1 und der Fettsäuren FA 18:0 und FA 20:0, die sich gut als Biomarker eigenen, wobei letztere ausschließlich in der Gruppe der Patienten mit metabolischem Syndrom mit einem signifikanten Anstieg auf den OFTT reagiert. Ähnlich verhält es sich mit dem sich auf den Organismus ungünstig auswirkenden SPM mit dem Acyl-Rest 16 [119], die mit einem Anstieg in der Gruppe mit metabolischem Syndrom auf den OFTT reagiert. Wie die antioxidativen Plasmalogene sind auch die protektiven SPM 24:0 [119] im metabolischen Syndrom basal herabreguliert und gleichzeitig zeigen sich erniedrigte Werte von SPM 22:4 und seinem Elongationsprodukt 24:4, die auf eine verminderte Transformation von der proinflammatorischen AA in diese hinweisen. Als Marker für einen chronisch postprandialen Zustand kommen vor allem diese Metaboliten in Frage, die in der Gruppe mit metabolischen Syndrom basal erhöht und keine oder eine nur abgeschwächte Reaktion auf den OFTT zeigen wie ApoB-48, PC 40:2. Der Anstieg in der Kontrollgruppe von protektiven PC 32:1 und FA 16:1 (n-7) unterstützen unsere These vom chronisch gesättigtem Status eines Patienten mit metabolischem Syndrom und von veränderter Enzymaktivität mit verringerter SCD-1 Aktivität. Weiter wiesen unsere Probanden im Vergleich zu der Kontrollgruppe laborchemisch eine chronische Entzündung auf, gekennzeichnet durch basal erhöhte Leukozytenzahl, erhöhten SuPar-Wert und CRP-Wert sowie erhöhte Werte von proinflammatorischen ω-6-Fettsäuren im Serum. Trotz des auch erhöhten Basalwertes von CRP, verzeichnete sich postprandial in der metabolischen Syndrome Gruppe eine nicht zu erklärende Senkung der CRP-Werte, die bei Gesunden nicht beobachtet werden konnte. Auch die Konversion von PC-Spezies in LPC-Spezies bei der verschiedene LPCA Subtypen mit Substratspezifität [183] verantwortlich sind, weisen unterschiedliche Ausprägung bzw. Effizienz in den verschieden Gruppen auf. Z.B. nimmt die Konzentration von LPC 18:3 und der Fettsäure 14:0 zu, während PC 32:3 in der Kontrollgruppe abnimmt, in den anderen Gruppen aber nicht. Die Ergebnisse weisen auf unterschiedliche Konzentrationen der LPCA in den unterschiedlichen Gruppen hin. Weitere Enzyme, die in den Gruppen unterschiedlich hoch vorliegen, sind SCD, wie sich an der höheren Konzentration der protektiven PC Spezies 32:1 und PC 32:2 zeigt, die Desaturationsprodukte von SCD enthalten [119] und erhöhte Werte in der Gruppe mit metabolischen Syndrome aufweisen und in dieser reaktiv auf den OFTT ansteigen. Adiponektin, Ghrelin und Leptin wurden als Marker Zytokine für die Regulation im Fettgewebe bestimmt und wiesen als Zeichen einer chronischen Adaption des Fettgewebes an den chronischen postprandialen Status mit konsekutiver Veränderung der Regulation veränderte Werte im Vergleich zur Kontrollgruppe auf. Serum Kreatinin und

FGF23 waren in der metabolischen Gruppe signifikant höher, auch wenn man FGF23 bei nierengesunden Nichtdiabetikern bestimmte.

7 Appendix

7.1 Abbreviations

Abbreviation	Description
AA	Arachidonic Acid
ACC	Acetyl Coenzyme A carboxylase
ACE	Angiotensin Converting Enzyme
AChE	Acetyl Cholinesterase
ACOX	Acyl-CoA oxidas
AHA	American Heart Association
AMPK	AMP-activated protein kinase
Apo	Apolipoprotein
AT	Adipose Tissue
ATP	Adenosine triphosphate
ATP III	Adult Treatment Panel III
ATX	Autotaxin
BMI	Body Mass Index
C	Celsius
C atom	Carbohydrate Atom
CAD	Cardiac Artery Disease
cAMP	Cyclic Adenonsin-Monophosphat
CarboH	Carbohydrate
CE	Cholesterylester
Cer	Ceramide
CHD	Coronary Heart Disease
CM	Chylomicron
Co	Control Group
co A	Coenzyme A
CPT-1	Carnitine palmitoyl transferase 1
CRP	C-reactive Protein
CVD	Cardiovascular Disease
D9D	Delta-9-Desaturase
DAG	Deutsche Adipositas Gesellschaft
DGAT	Diacylglycerol Acyltransferase
DHA	Docosahexaeoic Acid
DPA	Docosapentaenoic Acid
EDTA	Ethylendiamintetraacetat
EIA	Enzyme Immunoassay
ELISA	Enzyme Linked Immunosorbent Assay
ELVOL	Elongation Of Very Long Chain Fatty Acids Protein

EPA	Eicosapentaenoic Acid
ER	Endoplasmic Reticulum
ESI	Electrospray Ionization
eV	Electron Volt
FA	Fatty Acid
FABP	Fatty-acid-binding proteins
FAD	Fatty Acid Desaturase
FAME	Fatty Acid Methyl Esters
FAS	Fatty acid synthase
FATP	Fatty-acid transport protein
FCH	Familial combined hyperlipidemia
fFA	Free Fatty Acids
FGF-23	Fibroblast Growth-Factor 2
G	Gramm
GC-MS	Gas chromatography
GISIS	Glucose-Stimulated Insulin Secretion
GLUT	Glucose transporter
GPAT	Glycerol-3-phosphate acyltransferase
GPI	Glycosylphosphatidylinositol
H	Hour
HB	Hemoglobin
HDL	High Density Lipoprotein
HLP	Hyperlipidemia
HMW	High molecular weight
HPTLC	High Performance Thin Layer Chromatography
HRP	Horseradish
HTS	High Throughoutput Screening
ICAM	Intercellular Adhesion Molecule
IDF	International Diabetes Federation
IDL	Intermediate Density Lipoprotein
IKKβ	Inhibitor of Nuclear Factor Kappa-B Kinase Subunit Beta
IL	Interleukin
Kcal	Kilo Calorie
Km	Michaelis Konstante
L	Liter
LC	Liquid Chromatography
LCAS	Long chain acyl-CoA synthetase
LCAT	Acyltransferase
LDL	Low Density Lipoprotoein
Li.Hep	Lithium Heparin
LPA	Lysophosphatidic Acid
LPC	Lysophosphatidylcholine

LPCAT	Lyso-PA Acyltransferase
LPL	Lipoprotein Lipase
LPS	Lipopolysaccharide
LPS	Lysophosphatidylserine
LVH	Left ventricular Hypertrophy
M	Meter
M	Mol/L
m/z	Mass-To-Charge Ratio
Max	Maximal
MetSyn	Metabolic Syndrome
MGAT	Monoacylglycerol Acyltransferase
Min	Minute
mmHg	Mm Quecksilbersäule
MS	Mass Spectrometry
MSH	Melanocyte-Stimulating Hormones
MUFA	Monounsaturated Fatty Acids
N	Normal = Molar
N	Number
NCEP	National Cholesterol Education Program
NEFA	Not Esterified Fatty Acids,
NF-kB	Nuclear Factor (NF)- kB Transcription
Ng	Nanogramm
NHLBI	National Heart, Lung and Blood Institute
OFTT	Oral Fat Tolerance Test
OGTT	Oral Glucose Tolerance Test
P	Probability
PAI	Plasminogen-Activator-Inhibitor,
PE	Phosphatidylethanolamine
PE(P-	Plasmalogen
PepT	Peptide transporter
PHMB	Polyhexamid
PMN	Polymorphonuclear Leukocytes
PPAR	Peroxisome Proliferator-Activated Receptors
PROCAM	Prospective Cardiovascular Münster
PS	Phosphatidylserin
PTFE	Polytetrafluoroethylene
PTV	Programmed Temperature Vaporizer
PUFA	Polyunsaturated Acids
R	Risk Group
ROS	Reactive oxygen species
RR	Blood pressure
RXR	Retinoid X Receptor

SCD	Stearoyl-CoA desaturase
SD	Standard Deviation
SE	Standard Error
SGLT	Sodium-glucose linked transporter
SHP	Small Heterodimer Partner
SIM	Selected Ion Monitoring
SPM	Sphingomyelin
SREBP2	Sterol Regulatory Element-Binding Protein
SRM	Selected Reaction Monitoring
SUFA	Saturated Fatty Acids
SuPar	Soluble
SuPar	Soluble Urokinase-Type Plasminogen Activator Receptor
t	Time
T2D	Type 2 diabetes
TAG	Triglyceride
TLC	Thin layer chromatography
TMB	Tetramethylbenzidin
TNFα	Tumor Necrosis Factor
UCP	Uncoupling Protein
UKR	Universitätsklinikum Regensburg
UPAR	Urokinase-Type Plasminogen Activator Receptor
V	Velocity
V	Volt
V/V	Volume/Volume
VLDL	Very Low Density Protein
VSMC	Vascular Smooth Muscle Cell
W/H	Waist-To-Hip-Ratio
WHO	World Health Organisation

7.2 Questionnaire

Patient: Auswerteschlüssel ein/aus

Name: xx Vorname: xy
Geburtsdatum: 01.01.1500 Geschlecht: m
Patientennummer: 84000104 Studie: Basic questionnaire

Wichtig: Bitte zuletzt ganz unten auf WEITER klicken um Daten in die Datenbank abzuspeichern!

Inhaltsverzeichnis

ALLGEMEINE ANAMNESE

1.1 Diabetes

- klassische Diabetes mellitus-Symptome + Nicht-Nüchtern-Blutzucker* >= 200 mg/dl *oder*
- Nicht-Nüchtern-Blutzucker* >= 200mg/dl an 2 Tagen *oder*
- Nüchtern-Blutzucker* >= 12 mg/dl an 2 Tagen *oder*
- pathologischer oraler Glukose-Toleranz-Test *oder*
- Applikation von Insulin oder Antidiabetika.

* Messung von venösem Blut

	Nein	Ja	Seit wann (Jahr : z.B. "2005")	Unbekannt
Typ 1	○	○		○
Typ 2	○	○		○

	Nein	Ja		Unbekannt
Brachten Sie ein sehr kleines bzw. sehr großes Kind auf die Welt?	○	○	Geburtsgewicht (g):	○
Hatten Sie eine Fehlgeburt?	○	○	Anzahl	○

1.2 Diabetische Endpunkte und assoziierte Erkrankungen

	Nein	Ja	Seit wann (Jahr : z.B. "2005")	Unbekannt
Hypertonie (einmalig RRsys>=140 mmHg *oder* einmalig RRdia >= 90 mmHg *oder* antihypertensive Therapie *oder* Diagnose eines arteriellen Hypertonus)	○	○		○
Koronare Herzerkrankung				
Koronarer Bypass	○	○		○
Ballondilatation	○	○		○
Myokardinfarkt	○	○		○
PAVK				
Gehstrecke über > 200m unter Schmerzen	○	○		○
Femoraler (od. ähnl.) Bypass	○	○		○
Femoraler (od. ähnl.) Stent	○	○		○
Zerebrovaskuläre Erkrankungen				
TIA oder Apoplex	○	○		○
Wurde an der Halsschlagader ein Eingriff vorgenommen?	○	○		○
Venöse Thrombose* **durch Ultraschall oder Kontrastmittel gesichert**	○	○		○
Lungenembolie*	○	○		○
Arterielle Embolie* *anschließend Marcumar-Einnahme	○	○		○
Periphere Neuropathie	○	○		○
Nephropathie Bekannte Nierenerkrankungen *oder* erhöhter Kreatininwert *oder* Dialysepflichtigkeit.	○	○		○
Retinopathie Laserung der Netzhaut	○	○		○
Dyslipidämie erhöhte Cholesterin- *oder* Triglyzeridwerte *oder* lipidsenkende Medikamente	○	○		○
Periodontitis	○	○		○
Demenz Gedächtnisstörungen Vergeßlichkeit	○	○		○

2.0 Körpermaße

(Falls der Wert nicht bekannt ist geben sie bitte das Zeichen - ein!)

Gewicht (kg)	

Größe (cm)	
Taillenumfang (cm) (Mitte zw. unterster Rippe u. Darmbeinschaufeloberrand)	
Hüftumfang (cm) (Trochanterenhöhe)	

3.0 Lebensführung und Ernährung

Sind Sie mindestens 3x/Woche über mehr als 30 Minuten am Stück einer mittleren oder schweren körperlichen Belastung ausgesetzt?	○ Nein	○ Ja	○ Unbekannt			
Wie oft verzehren Sie Gemüse oder Obst/dunkles Brot (Roggen, Vollkorn)?	○ täglich	○ 1-3x/W.	○ manchmal	○ nie	○ Unbekannt	
Wie oft nehmen Sie Alkohol zu sich?	○ täglich	○ 1-3x/W.	○ manchmal	○ nie	○ Unbekannt	
Rauchen Sie? Wenn ja, wieviele Zigaretten am Tag?	○ Nein	○ 1-10	○ 11-20	○ >20	○ Unbekannt	
Haben Sie jemals über längere Zeit geraucht? Wenn ja, wann haben Sie aufgehört?	○ Nein	○ Ja	Since when (Year: p.e. "2005")?	○ Unbekannt		
Wurde jemals ein erhöhter Blutzucker festgestellt?	○ Nein	○ Ja	○ Unbekannt			

4.0 Dauermedikation

Medikament	Nein	Ja	Seit wann (Jahr : z.B. "2005")	Unbekannt
Antidiabetika	○	○		○
Insulininjektion	○	○		○
Oral	○	○		○
Lipidsenker	○	○		○
CSE-Hemmer	○	○		○
Fibrate	○	○		○
Nicotinsäurederivate	○	○		○
Lipasehemmer	○	○		○
Anionenaustauscher	○	○		○
Absorptionshemmer	○	○		○
Anti-Hyperurikämika	○	○		○
Antihypertensiva	○	○		○
Calciumantagonisten	○	○		○
Betarezeptorenblocker	○	○		○
ACE-Hemmer	○	○		○
Alpha - Rezeptorenblocker	○	○		○
Angiotensin-II-Rezeptor-Antagonisten	○	○		○
Diuretika	○	○		○
Sonstige	○	○		○
Medikamente zur Behandlung der Herzinsuffizienz	○	○		○
Medikamente zur Behandlung der koronaren Herzerkrankung	○	○		○
Orale Antikoagulantien	○	○		○
Thrombozytenaggregationshemmer	○	○		○
Sexualhormone (Pille, Menopause, etc.)	○	○		○
Antipsychotika	○	○		○
Schilddrüsenmedikamente	○	○		○
Längerfristige Schmerzmedikation	○	○		○
	Nein	Ja	Welches Medikament	Unbekannt
Musste ein Medikament aufgrund einer Unverträglichkeit abgesetzt werden?	○	○		○

5.0 Familienanamnese

(Falls ein Datum nicht bekannt ist geben sie bitte das Zeichen - ein!)

	Geburtsjahr	Sterbejahr (falls verstorben)	Alter
Vater (I)			
Mutter (II)			
Großvater (I)			
Großmutter (I)			
Großvater (II)			
Großmutter (II)			

	Anzahl	Anzahl verstorben vor dem 60. LJ
Brüder		
Schwestern		
Söhne		
Töchter		

5.1 Diabetes, diabetische Endpunkte und assoziierte Erkrankungen bei Familienangehörigen

(falls zutreffend bitte ankreuzen, optional)

	Eltern	Großeltern mütterlich	Großeltern väterlich	Geschwister	Kinder	Partner (leiblicher Vater/ Mutter der Kinder)	Familie des Partners
Diabetes Typ I	☐	☐	☐	☐	☐	☐	☐
Diabetes Typ II	☐	☐	☐	☐	☐	☐	☐
Hypertonie	☐	☐	☐	☐	☐	☐	☐
KHK	☐	☐	☐	☐	☐	☐	☐
PAVK	☐	☐	☐	☐	☐	☐	☐
Zerebrovaskuläre Erkr.	☐	☐	☐	☐	☐	☐	☐
Thrombosen/Embolien	☐	☐	☐	☐	☐	☐	☐
Periphere Neuropathie	☐	☐	☐	☐	☐	☐	☐
Nephropathie	☐	☐	☐	☐	☐	☐	☐
Dyslipidämie	☐	☐	☐	☐	☐	☐	☐
Periodontitis	☐	☐	☐	☐	☐	☐	☐
Demenz	☐	☐	☐	☐	☐	☐	☐
Adipositas	☐	☐	☐	☐	☐	☐	☐
Retinopathie	☐	☐	☐	☐	☐	☐	☐

7.3 CURRICULUM VITAE

Personal data

Name: Kaviany, Afssun

Address: Prinzenweg 27, 93047 Regensburg

Mobile: 0176 70 140773

E-mail: Sonnenblumekaviany@googlemail.com

Nationality: German

Date of birth: 12.12.1982

Education and professional experience

08/1989 - 06/2002	College
10/2002 - 05/2003	Chemistry student at Munich University
05/2003 - 08/2003	Internship at “Klinikum Schwabing” hospital (Munich)
09/2003 - 09/2004	Instructive in First Aid at Malteser
09/2004 - 09/2005	Medical student in Strasbourg
09/2005 - 09/2006	Medical student at Luxembourg University
since October 2006	Medical student at Regensburg University
Summer 2008	Clinical elective at Giessen Hospital
02/2009 - 04/2009	Clinical elective in Cameroon
09/2010 – 02/2011	Medical student in Prague (2nd faculty)
Since January 2013:	“Barmherzige Brüder“ hospital

7.4 Presentation

Poster presentation in Vienna at the workshop “Lipid droplets and endolysosomes as dynamic organelles of lipid deposition and release”, July 9-11, 2012.

7.5 Grants

"The research leading to these results has received funding from the European Community's Seventh Framework Program (FP7/2007-2013) under grant agreement n° 202272, IP-Project LipidomicNet".

7.6 Acknowledgements

This work has been the most challenging experience in my academic education so far. It could only be completed with the support and patience of the following people, to whom I want to express my deepest gratitude. I thank Dr. Stefan Wallner for his guidance and patience as my supervisor despite his many other academic duties. He helped me to develop a statistical and scientific background over the last years. Dr. Evelyn Orso and Prof. Schmitz, who made this work possible by inspiring me with their great wisdom and challenged me with their high standards, while being very patient with me at the same time. I thank all the laboratory members, who supported the organization of this large study, and the MTA, who assisted in the laboratory work. I especially want to thank Daniela Biermeir, Helga Friedmann, Daniela Waagenknecht and Helga Staudner, who provided a nice atmosphere and gave me good wishes, organizational support and commitment whenever I needed them. I thank Dr. Liebisch and Markus Solleder, who were always ready to answer questions about lipids or IT problems. I thank Tatjana Konovalova, who helped me with the correlation data.

I thank the other doctoral candidates Ralph and Manuela Ayala Jurado, who participated in this project and with whom I recruited the volunteers and went through good and bad times together. My research would have been very lonely without them.

The 70 volunteers, without whom this work would not have been possible, who came to the clinic and stayed there for one whole day in order to let us collect their blood without receiving any payment.

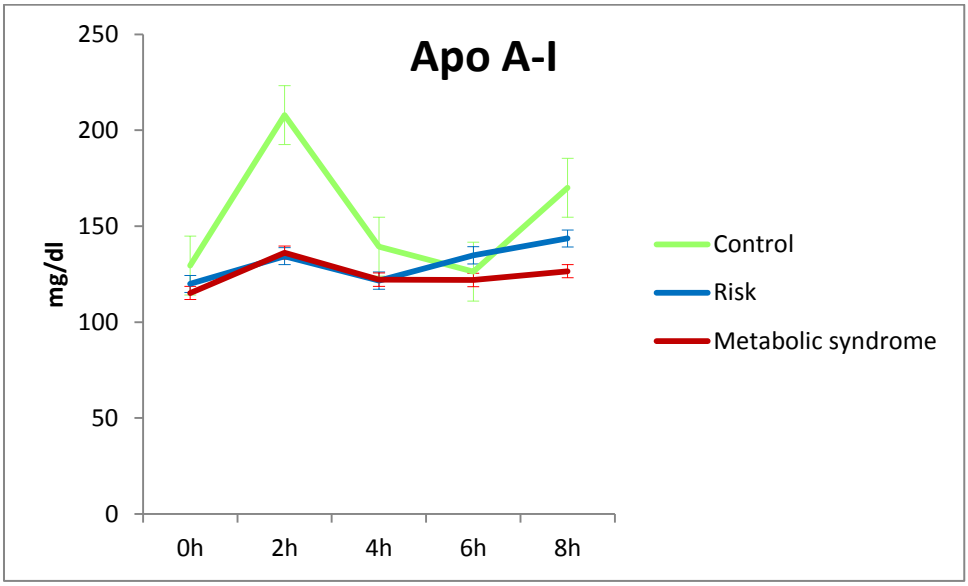
I thank my friends for relinquishing me for five years, especially my best friends Kyra Fichtner and Frauke Wichmann, whom I could only meet very rarely in my sparse spare time because of the far distance, and those friends who read through my thesis and gave me

feedback on my English in the form of proofreading: Lukas, Andreas Obergruschtberger, Jonas, Heike Oldenwurtz and Sonja Dormann.
I thank my boyfriend Florian, who supported me during difficult times, encouraged me and made so many compromises and sacrifices. When I wanted to go out because of the nice weather, he told me that I wanted to finish my thesis and that I would have the rest of my life to enjoy the weather. He supported me mentally and in the household to allow me to focus on this work. Without his love and energy, I would not have come this far.

I thank my family, without whom this thesis would not have been possible, who have supported me my whole life, who motivated me with their words and through their own success in life and who believed in me, even when I thought I would never finish this thesis. I especially thank my sister, who enriched this thesis with her aesthetic competence, helpful criticism and suggestions concerning the layout of this thesis
.

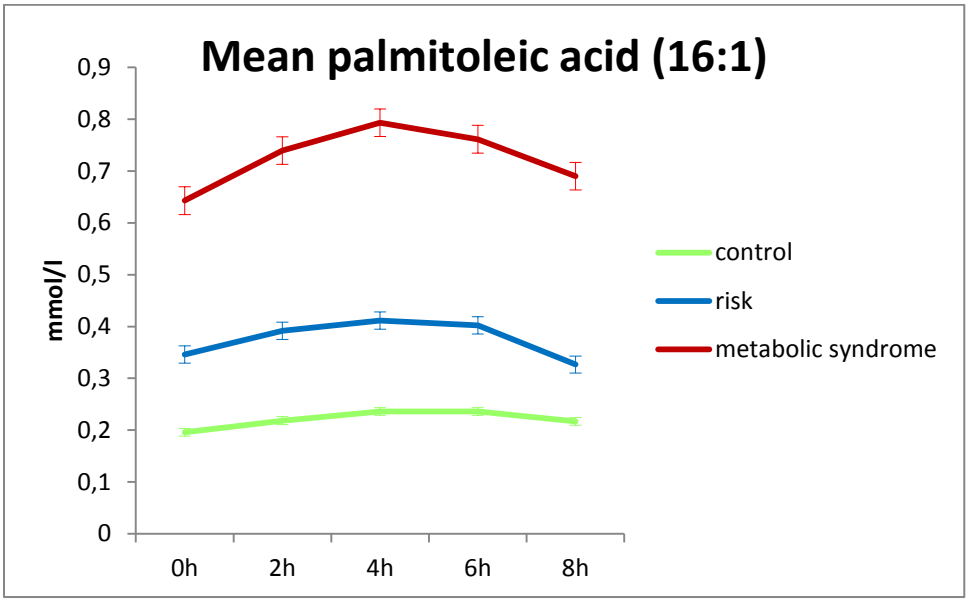
7.7 Supplement

7.7.1 Apo A-I



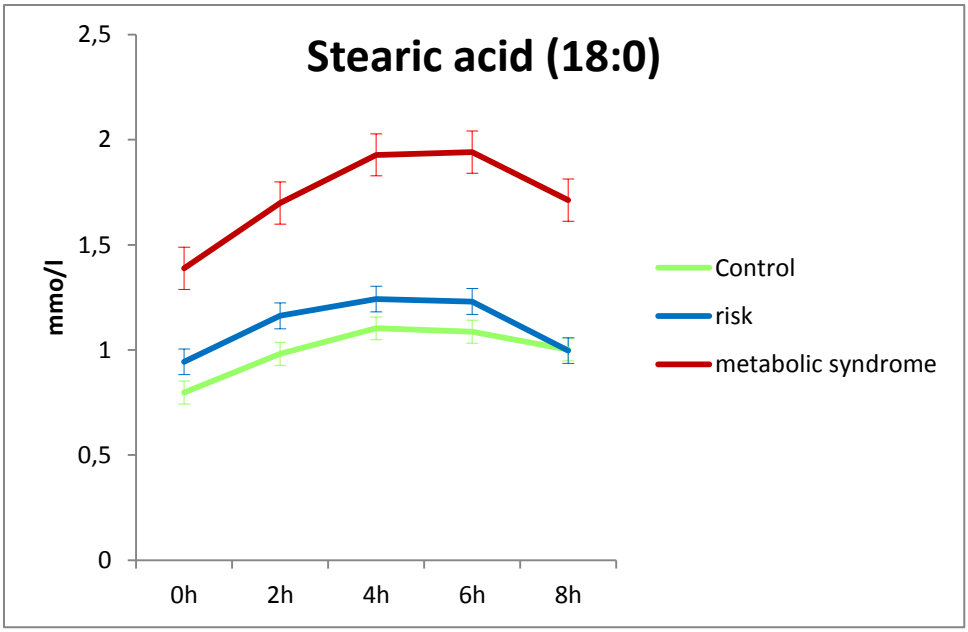
S 1: Apo A-I showed no significant difference over time in any of the groups p (control)=0.057; p (risk)=0.071; p(metabolic syndrome)=0.4, data is represented as means ± 1SE.

7.7.2 Fatty acids

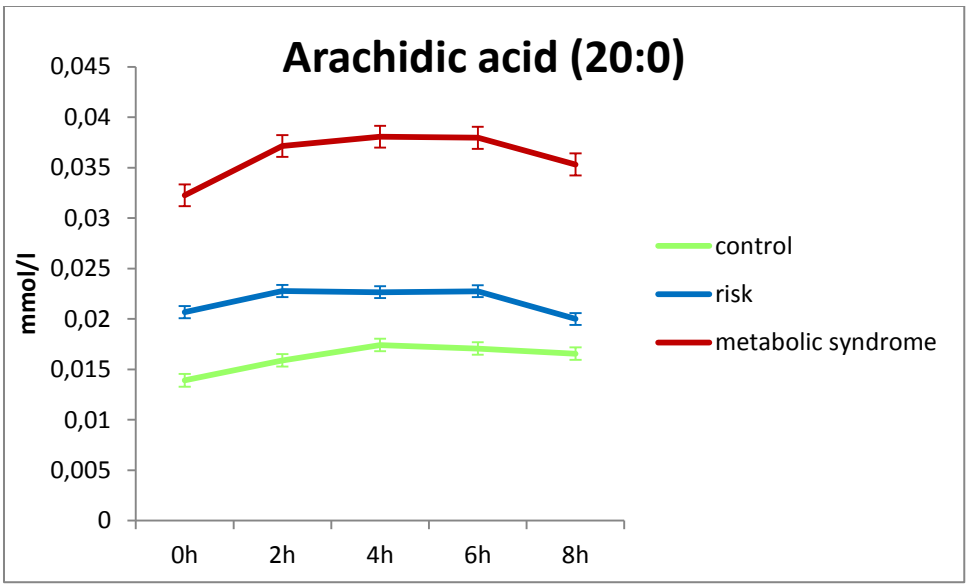


.

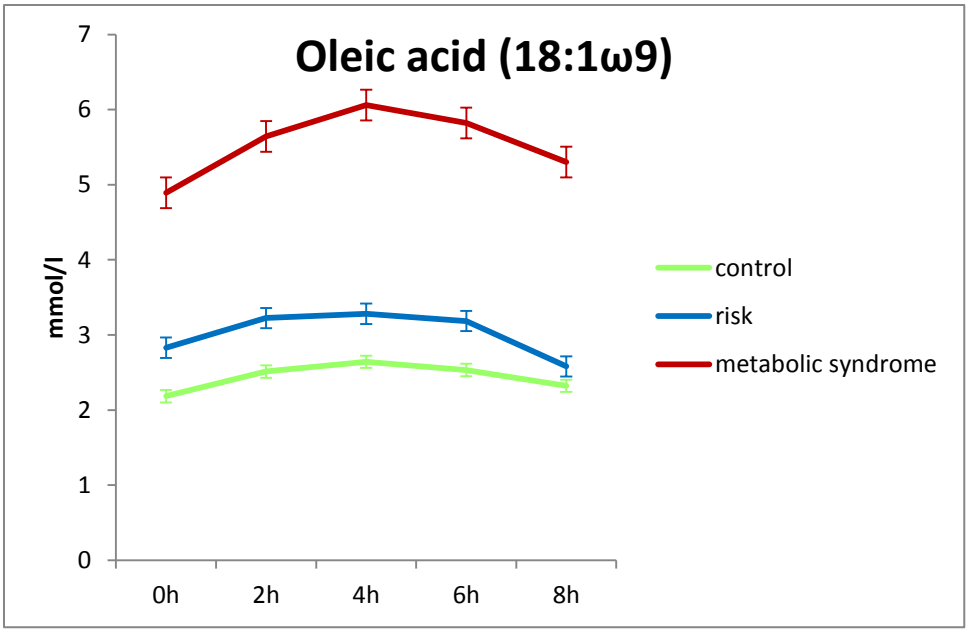
S 2: The comparison of the fatty acid 16:1(c-9) concentration of the groups at time zero was calculated using the Kruskal-Wallis test n=13 (control); n=20 (risk); n=33 (metabolic syndrome). Changes over time were evaluated using the Friedman test: n=12 (control) p=0.07; n=20 (risk) p<0.001; n=32 (metabolic syndrome), p<0.001. Data is represented as means ± 1SE.



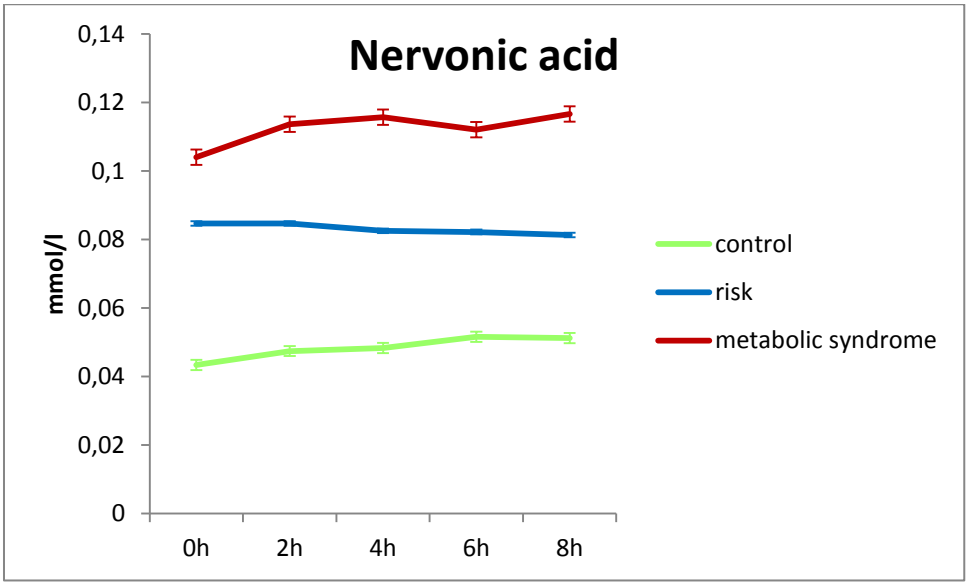
S 3: This graph shows fatty acid 18:0. The significance of the differences between the groups at time zero was calculated using Kruskal-Wallis: p=0.048, n=14 (control); n=20 (risk) n=33 (metabolic syndrome). Changes over time were tested by Friedman, p<0,001, n=12 (control); n=20 (risk); n=32; (metabolic syndrome). Data is represented in means ± 1SE.



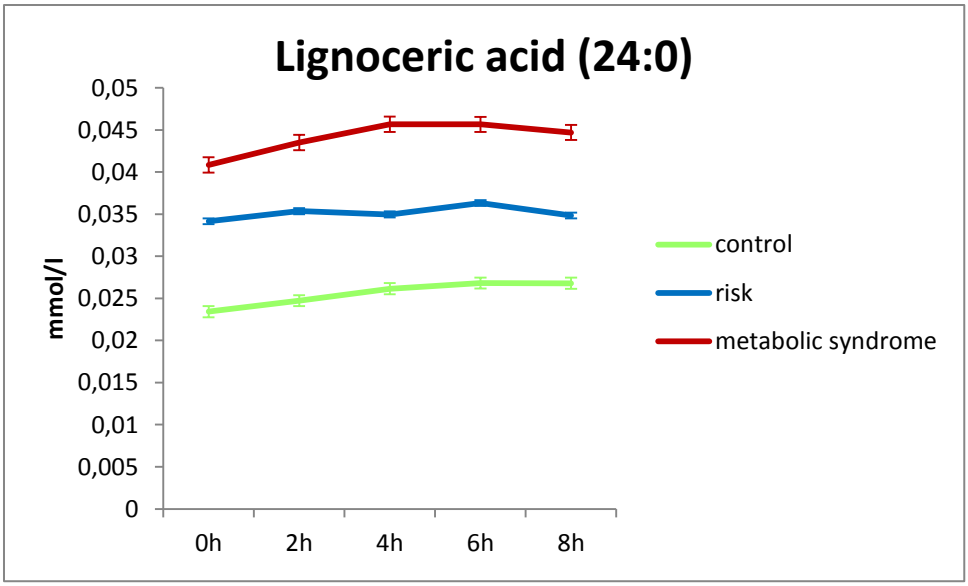
S4: Significantly different levels of arachidic acid between the groups (Kruskal-Wallis p=0.019). Changes over time were tested by Friedman n=12 (control), p=0.113; n=20 (risk) p=0.004; n= 32; (metabolic syndrome) p<0.001. Data is represented as means ± 1SD.



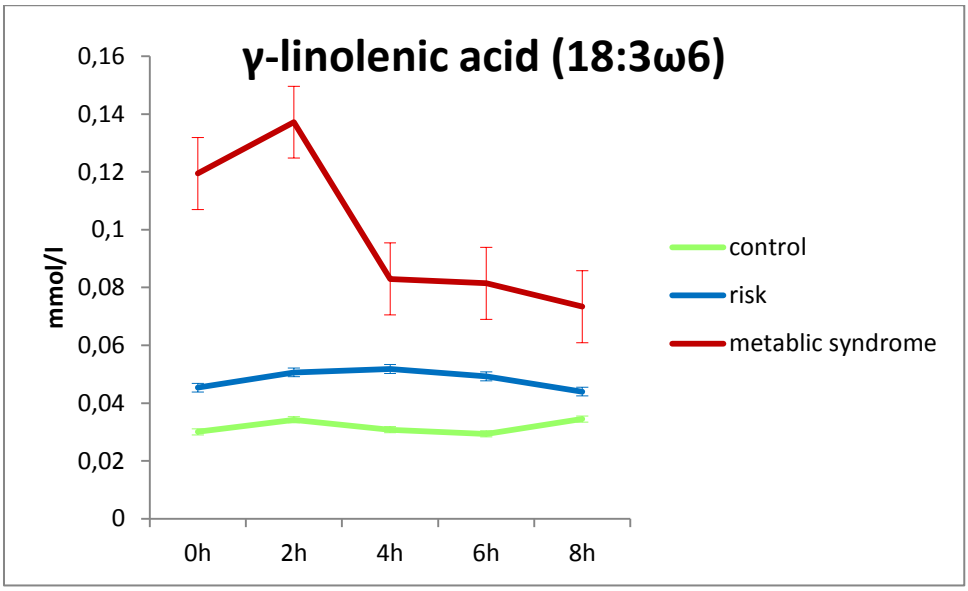
S 5: Oleic acid, significant differences between the groups, Kruskal-Wallis, p=0.01, and dynamics over time (Friedman-test, p=0.01, control, n=12; p<0.001 risk, n=20, p<0.001 metabolic syndrome, n=32). Data is represented as means ± 1SD.



S 6: Nervonic acid (Mann-Whitney U, p<0.001). Over time, there was no significant change in any of the groups (Friedman, p=0.19, control, n=12; p=0.87, risk, n=20, p=0.17, n=32, metabolic syndrome). Data is represented as means ± 1SE.

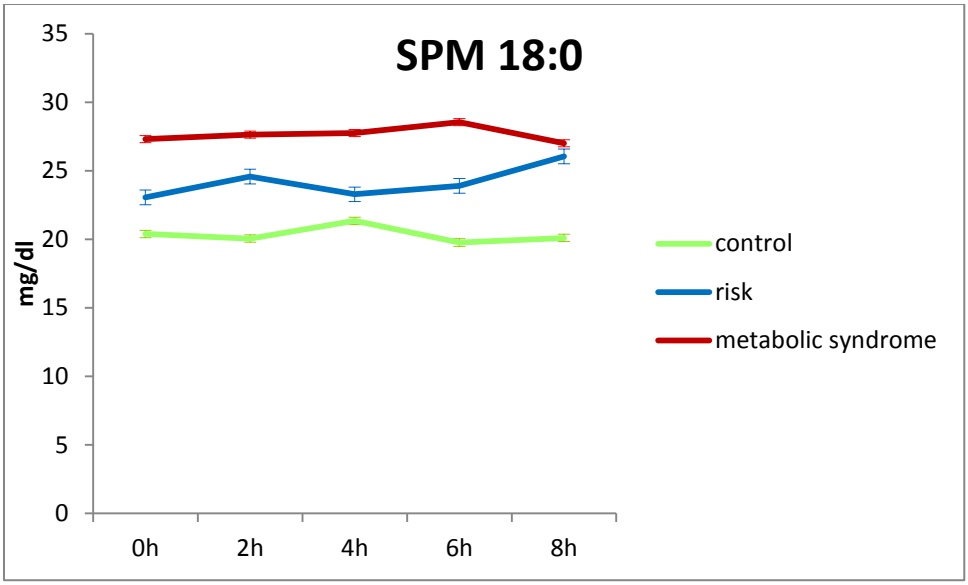


S 7: Lignoceric acid, Kruskal-Wallis, p=0.35, Friedman, p=0.29, control, n=12; p=0.7, risk, n=20, p=0.01, n=32, metabolic syndrome. Data is represented as means ± 1SE.

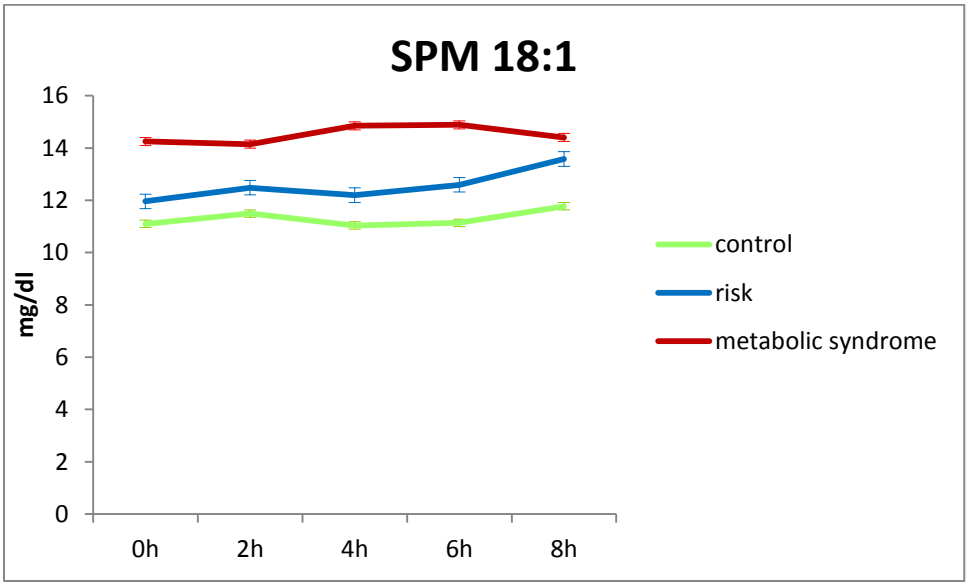


S 8: Friedman, p=0.6, control, n=12; p=0.01, risk, n=20, p< 0.001, n=32, metabolic syndrome. Data is represented as means ± 1 SE.

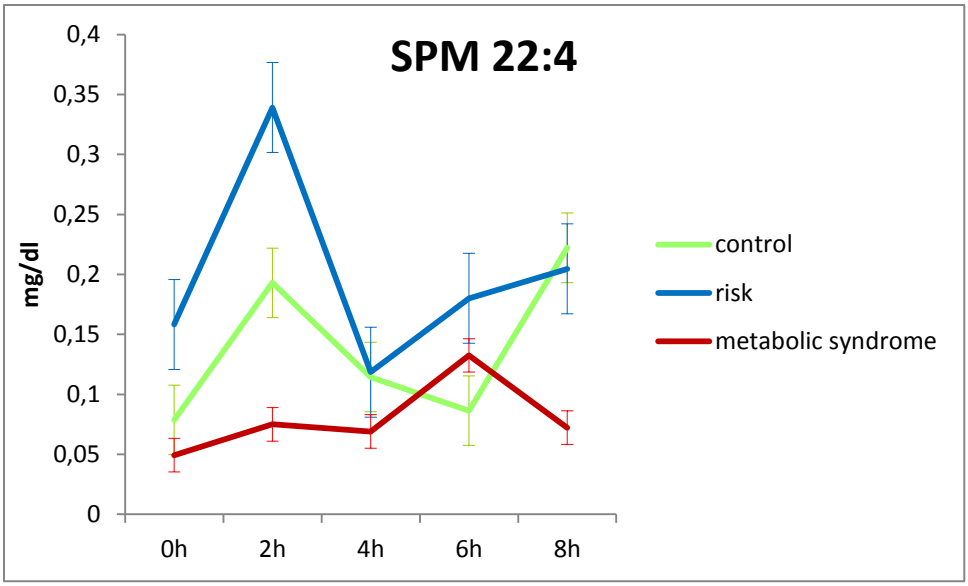
7.7.3 SPM



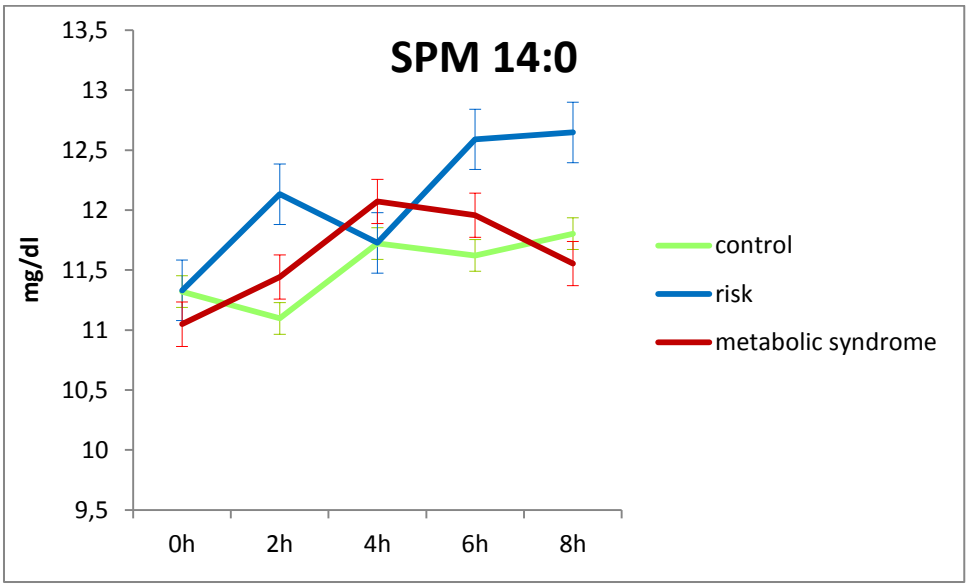
S 9: SPM 18:0, Kruskal-Wallis test: p=0.035, Friedman test p=0.27 control, p=0.004 risk, p=0.24 metabolic syndrome. Data is represented as means ± 1SE.



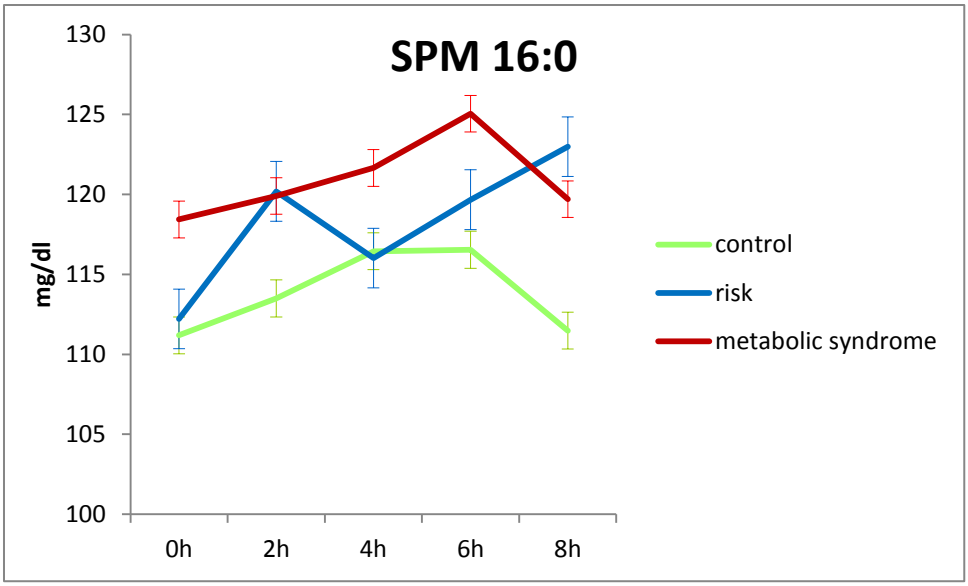
S 10: SPM 18:1, Kruskal-Wallis test: p=0.041, Friedman test p=0.488 control, p=0.251risk, p=0.28 metabolic syndrome. Data is represented as means ± 1SE.



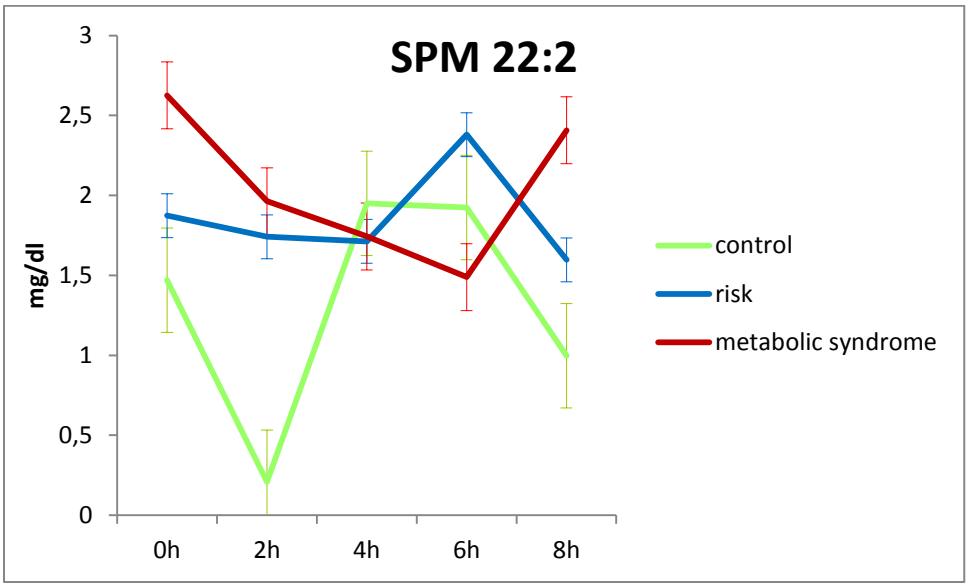
S 11: SPM 22:4, Kruskal-Wallis test p=0.015, Friedman test p=0.29 control, p=0.82 risk, p=0.12 metabolic syndrome. Data is represented as means ± 1SE.



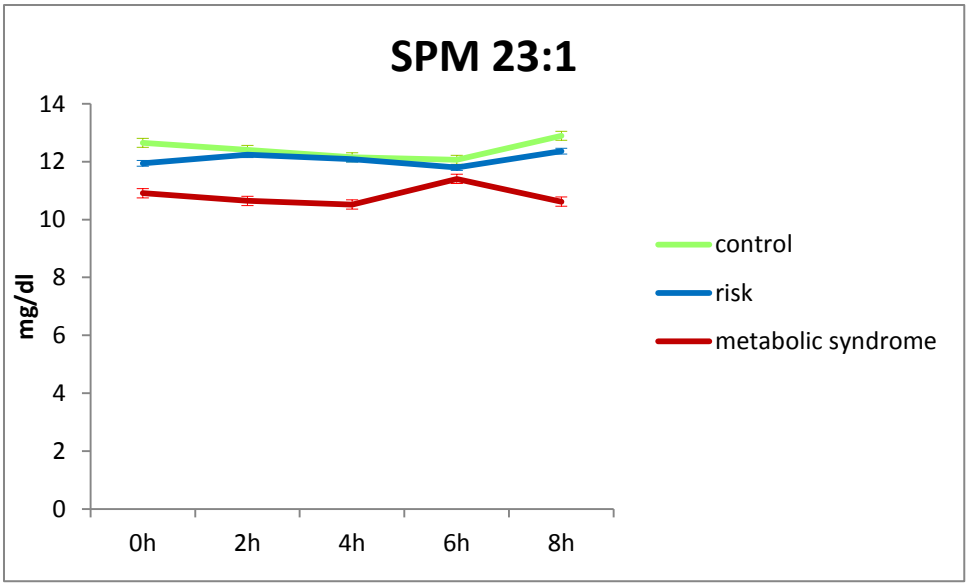
S 12: SPM 14:0, Kruskal-Wallis test: p=0.63, Friedman test p=0.49 control, p=0.075 risk, p=0.026 metabolic syndrome. Data is represented as means ± 1SE.



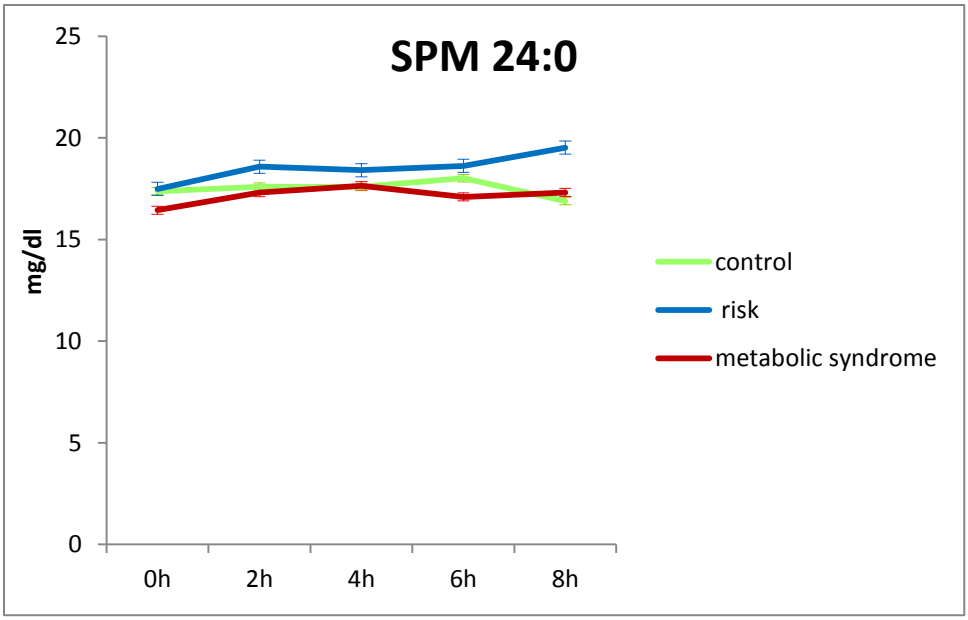
S 13: SPM 16:0, Kruskal-Wallis test 0.69; Friedman test: p=0.9 control p=0.15 risk, p=0.009 metabolic syndrome. Data is represented as means ± 1SE.



S 14: SPM 22:2 Kruskal-Wallis test p=0.23, Friedman test p=0.04 control, p=0.39 risk, p=0.024 metabolic syndrome. Data is represented as means ± 1SE.

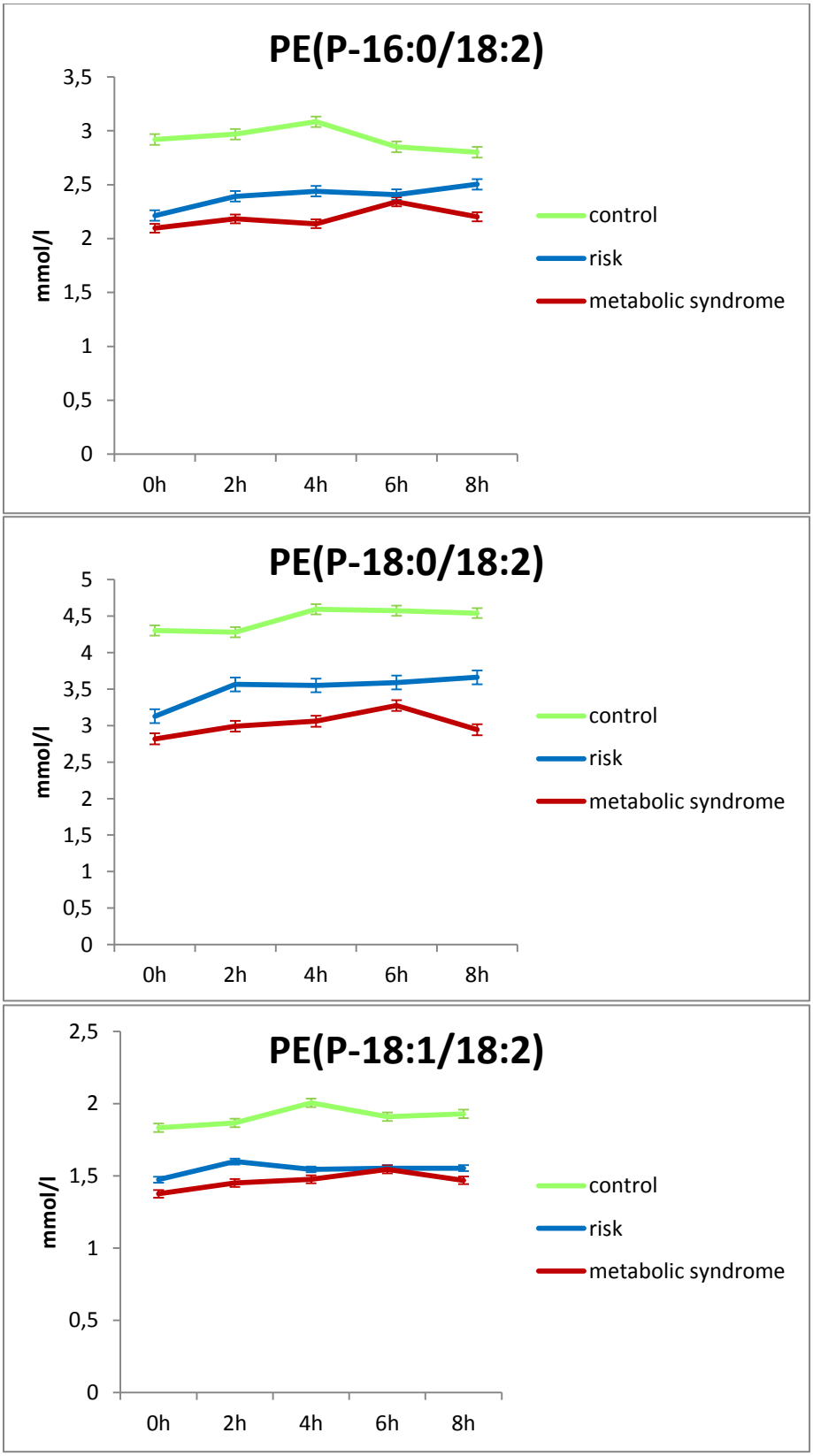


S 15: SPM 23:1 Kruskal-Wallis test p=0.164, Friedman test p=0.84 control, p=0.40 risk, p=0.016 metabolic syndrome Data is represented as means ± 1SE.

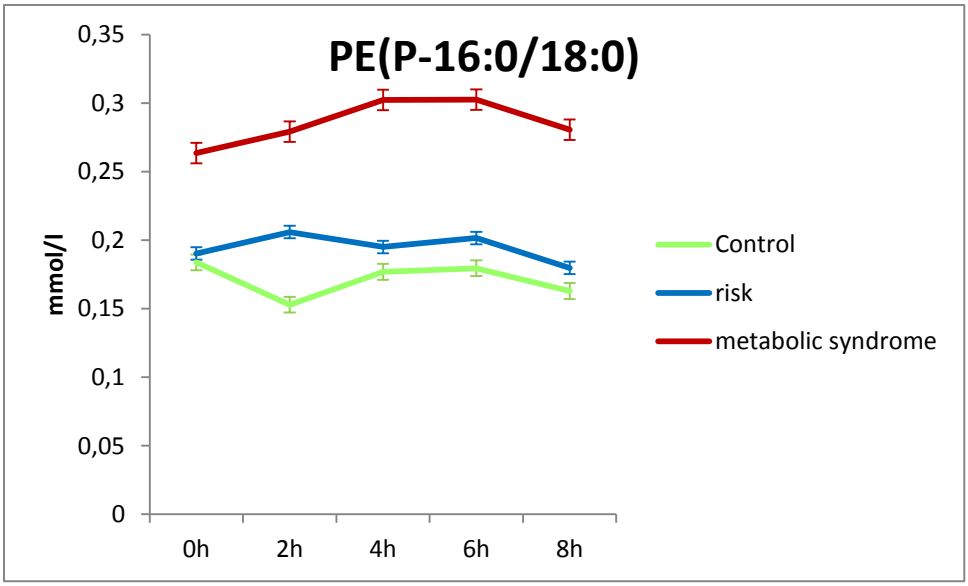


S 16: SPM 24:0; Kruskal-Wallis test p=0.37, Friedman test p=0.72 control, p=0.47 risk, p=0.003 metabolic syndrome. Data is represented as means ± 1SE.

7.7.4 Plasmalogens

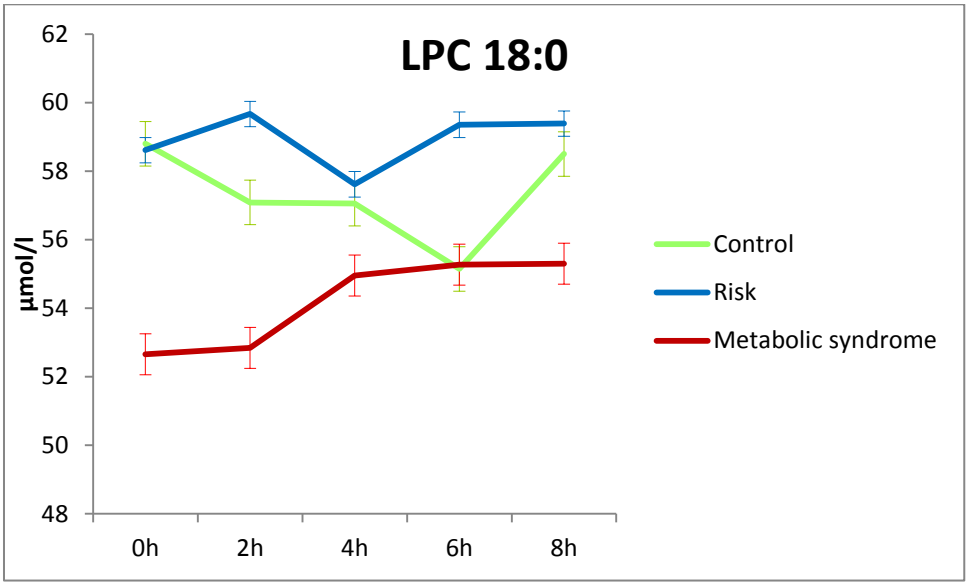


S 17: Sn-2: Linoleic acid: P(PE-16:0/18:2) Kruskal Wallis, p=0.07; Friedman, p=0.3, control; p=0.38, risk; p= 0.009, metabolic syndrome, P(PE-18:0/18:2) Kruskal Wallis, p=0.008 Friedman, p=0.6, control; p=0.07, risk; p=0.003, metabolic syndrome, P(PE-18:1/18:2) Kruskal-Wallis, p=0.037 Friedman, p=0.2, control; p=0.2 risk; p=0.3, metabolic syndrome. Data is represented as means ± 1 SE.

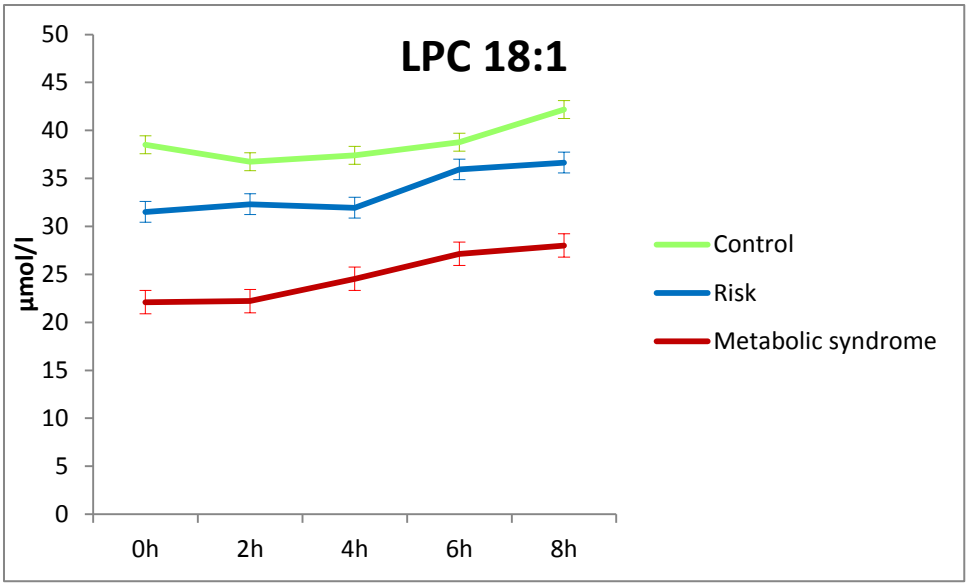


S 18: This picture depicts the plasmalogen 16:0/18:0. The differences between the groups were evaluated using the Mann-Whitney-U test n=13 control, n=31 metabolic syndrome.For changes over time, the Friedman test was used: n=12(control); n=18 (risk); n=30 (metabolic syndrome) There was only a change in the metabolic syndrome group (p=0.001). Data is represented as means ± 1SE

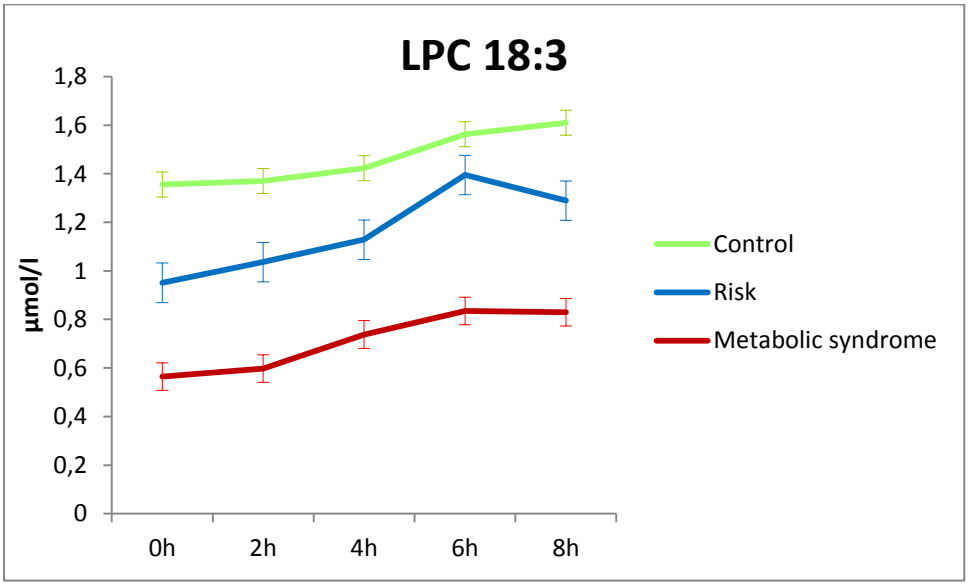
7.7.5 LPC



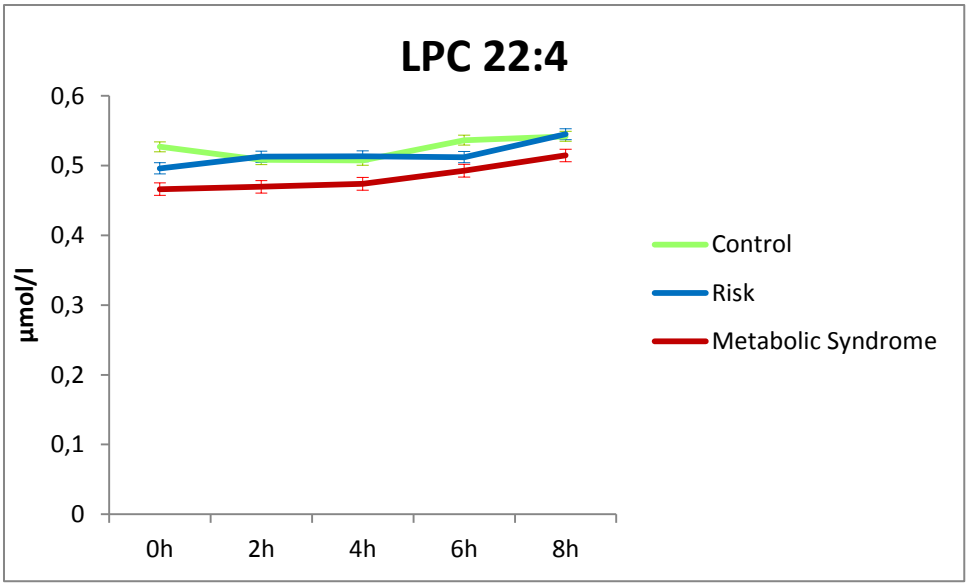
S 19: LPC 18:0: Kruskal-Wallis test: p=0.3, Friedman test p=0.58 control, p=0.3 risk, p=0.004 metabolic syndrome. Data is represented as means ± 1SE.



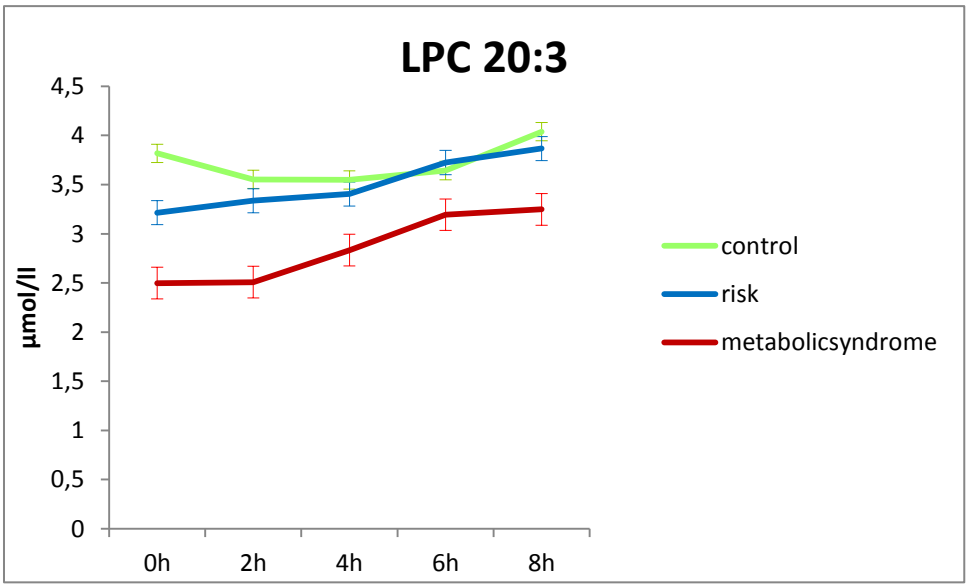
S 20: LPC 18:1, Kruskal-Wallis test: p<0.001, Friedman test p<0.001 control, p<0.009 risk, p<0.001 metabolic syndrome, data is represented as means ± 1SE.



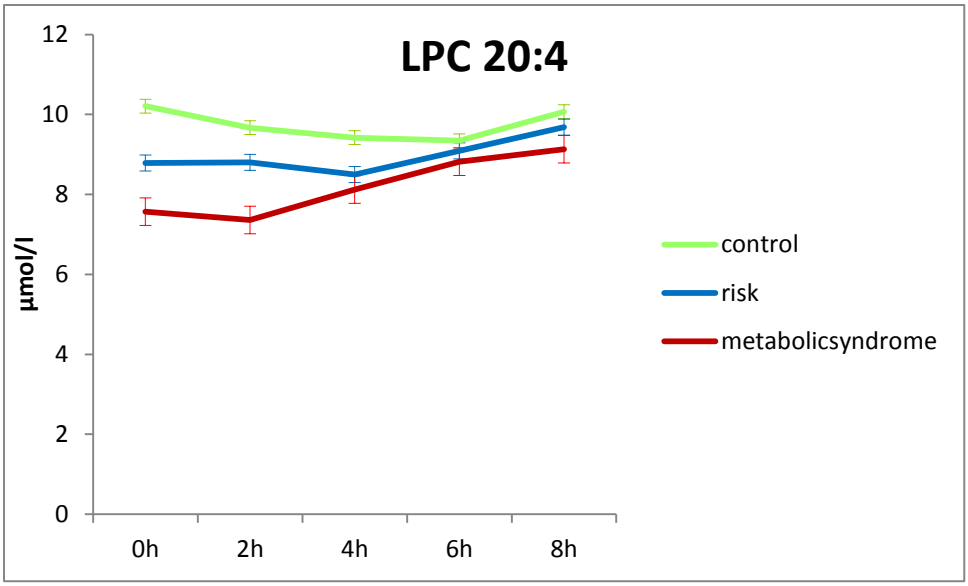
S 21: LPC 18:3, Kruskal-Wallis test: p<0.001, Friedman test p<0.001 control, p=0.002 risk, p<0.001 metabolic syndrome, data is represented as means ± 1SE.



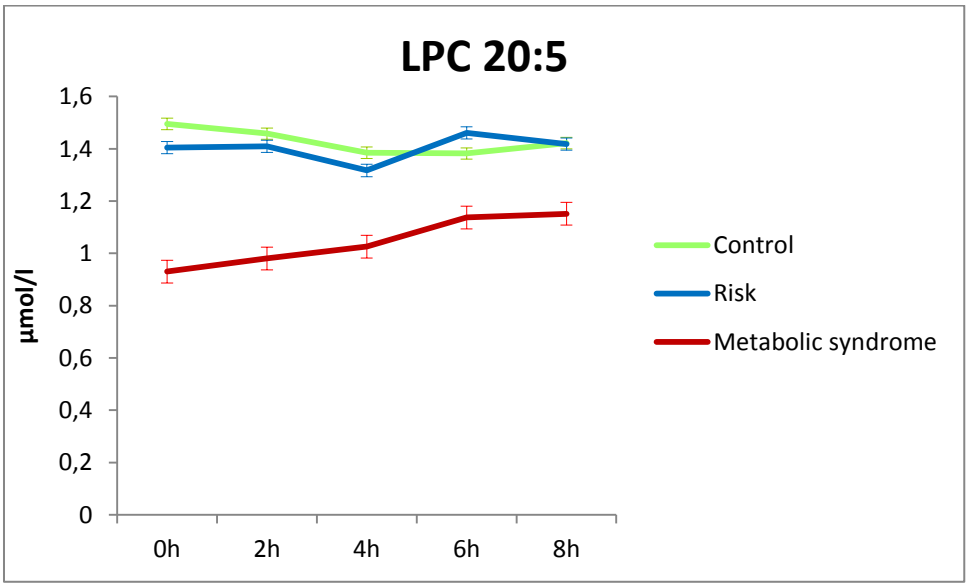
S 22: LPC 22:4, Kruskal-Wallis test: p=0.7, Friedman test p=0.007 control, p=0.51 risk, p=0.009 metabolic syndrome, data is represented as means ± 1SE.



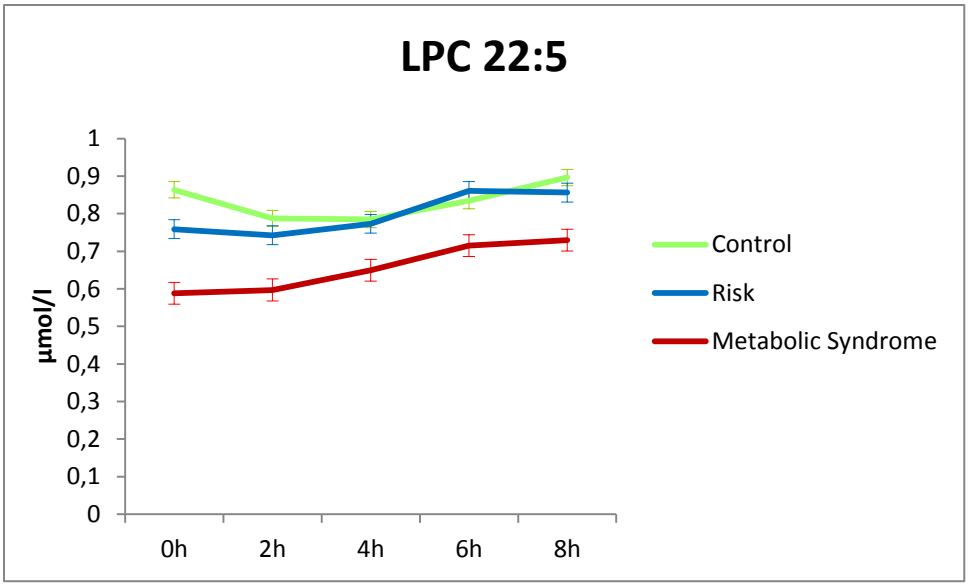
S 23: LPC 20:3, Kruskal-Wallis test: p=0.002 Friedman test p<0.001 control, p=0.13 risk, p<0.001 metabolic syndrome, data is represented as means ± 1SE.



S 24: LPC 20:4, Kruskal-Wallis test: p=0.009, Friedman test p<0.001 control, p=0.058 risk, p<0.001 metabolic syndrome, data is represented as means ± 1SE.



S 25: LPC 20:5, Kruskal-Wallis test: p=0.007, Friedman test p=0.007 control, p=0.51, p=0.009 metabolic syndrome, data is represented as means ± 1SE.



S 26: LPC 22:5, Kruskal-Wallis test: p<0.001, Friedman test, p<0.001 control, p=0.07 risk, p=0.001 metabolic syndrome, data is represented as means ± 1SE.

7.7.6 PC and LPC

	LPC 20:0	LPC 19:0	LPC 18:0	LPC 18:1	LPC 16:1	LPC 18:2	LPC 20:3	LPC 18:3	LPC 22:4	LPC 20:4	LPC 22:5	LPC 20:5	LPC 22:6
PC 40:7				22:6		22:5	20:4	22:4	18:3	**20:3**	18:2	**20:2**	18:1
PC 38:6				20:5	22:5	20:4	18:3	**20:3**		18:2	**16:1**	18:1	**16:0**
PC 40:6			22:6	22:5		22:4	**20:3**		18:2	**20:2**	18:1		**18:0**
PC 36:5					20:4	18:3		18:2	**14:1**	**16:1**	**14:0****	**16:0**	
PC 40:5	20:5		22:5	22:4			**20:2**		18:1		**18:0**	**20:0**	
PC 42:5	22:5									22:1	**20:0**	22:0	
PC 34:4					18:3		**14:1**	**16:1**	**12:0*****	**14:0****			
PC 36:4				18:3	**20:3**	18:2	**16:1**	18:1	**14:0****	**16:0**			
PC 38:4			20:4	**20:3**		**20:2**	18:1		**16:0**	**18:0**			
PC 40:4	20:4		22:4					22:1	**18:0**	**20:0**			
PC 42:4	22:4						22:1	24:1	**20:0**	22:0			
PC 32:3						**14:1**	**12:0*****	**14:0****					
PC 34:3					18:2	**16:1**	**14:0****	**16:0**					
PC 36:3			18:3	18:2	**20:2**	18:1	**16:0**	**18:0**					
PC 40:3	**20:3**					22:1	**20:0**	22:0					
PC 32:2				**14:1**	**16:1**	**14:0****							
PC 34:2				**16:1**	18:1	**16:0**							
PC 38:2	18:2		**20:2**		22:1	**20:0**							
PC 40:2*	**20:2**			22:1	24:1	22:0							
PC 30:1				**12:0*****	**14:0****								
PC 32:1			**14:1**	**14:0****	**16:0**								
PC 34:1	**14:1**		**16:1**	**16:0**	**18:0**								
PC 36:1	**16:1**		18:1	**18:0**	**20:0**								
PC 38:1	18:1			**20:0**	22:0								
PC 40:1			22:1	22:0	24:0								
PC 44:1	24:1												
PC 26:0													
PC 28:0			**10:0*****										
PC 30:0**	**10:0*****		**12:0*****										
PC 32:0	**12:0*****	13:0	**14:0****										
PC 34:0	**14:0****	**15:0***	**16:0**										
PC 36:0	**16:0**	**17:0**	**18:0**										
PC 40:0	**20:0**		22:0										
PC 42:0	22:0		24:0										

S 27: Control (4h), PC, LPC and FA level at 4 hours were compared to baseline value by calculating the difference between the mean at four hours and at starting point. Increased levels compared to baseline are highlighted in bold (red), decreased levels compared to baseline are highlighted in light grey (blue), and those with no significant changes black. In the middle boxes, those fatty acid species are shown from which the respective PC species could potentially be built with the corresponding LPC species (at the top). PC species are shown on the left. A significant decrease is defined as ≥ 1.2. Additionally, the significance we calculated with is shown in stars:: *<0.05, **<0:01, ***<0.001, (scheme based on description by Thomas Kopf).

	LPC 20:0	LPC 19:0	LPC 18:0	LPC 18:1	LPC 16:1	LPC 18:2	LPC 20:3	LPC 18:3	LPC 22:4	LPC 20:4	LPC 22:5	LPC 20:5	LPC 22:6
PC 40:7				22:6		22:5	20:4	**22:4**	18:3	**20:3**	18:2	20:2	18:1
PC 38:6				20:5	22:5	20:4	18:3	**20:3**		18:2	16:1	18:1	**16:0**
PC 40:6			22:6	22:5		**22:4**	**20:3**		18:2	20:2	18:1		**18:0**
PC 36:5					20:4	18:3		18:2	**14:1**	16:1	**14:0****	**16:0**	
PC 40:5	20:5		22:5	**22:4**			20:2		18:1		**18:0**	**20:0**	
PC 42:5	22:5									**22:1**	**20:0**	**22:0**	
PC 34:4*					18:3		**14:1**	16:1	**12:0****	**14:0****			
PC 36:4				18:3	**20:3**	18:2	16:1	18:1	**14:0****	**16:0**			
PC 38:4			20:4	**20:3**		20:2	18:1		**16:0**	**18:0**			
PC 40:4	20:4		**22:4**					**22:1**	**18:0**	**20:0**			
PC 42:4	**22:4**						**22:1**	**24:1**	**20:0**	**22:0**			
PC 32:3						**14:1**	**12:0****	**14:0****					
PC 34:3					18:2	16:1	**14:0****	**16:0**					
PC 36:3			18:3	18:2	20:2	18:1	**16:0**	**18:0**					
PC 40:3	**20:3**					**22:1**	**20:0**	**22:0**					
PC 32:2				**14:1**	16:1	**14:0****							
PC 34:2				16:1	18:1	**16:0**							
PC 38:2	18:2		20:2		**22:1**	**20:0**							
PC 40:2	20:2			**22:1**	**24:1**	**22:0**							
PC 30:1				**12:0****	**14:0****								
PC 32:1			**14:1**	**14:0****	**16:0**								
PC 34:1	**14:1**		16:1	**16:0**	**18:0**								
PC 36:1	16:1		18:1	**18:0**	**20:0**								
PC 38:1	18:1			**20:0**	**22:0**								
PC 40:1			**22:1**	**22:0**	**24:0**								
PC 44:1	**24:1**												
PC 26:0													
PC 28:0			**10:0*****										
PC 30:0**	**10:0*****		**12:0****										
PC 32:0	**12:0****	**13:0*****	**14:0****										
PC 34:0	**14:0****	**15:0***	**16:0**										
PC 36:0	**16:0**	**17:0**	**18:0**										
PC 40:0	**20:0**		**22:0**										
PC 42:0	**22:0**		**24:0**										

S 28: Control (6h), increased levels compared to baseline are highlighted in bold (red), decreased levels compared to baseline are highlighted in light grey (blue), and those with no significant changes black. In the middle boxes, those fatty acid species are shown from which the respective PC species could potentially be built with the corresponding LPC species (at the top). PC species are shown on the left. A significant decrease is defined as ≥ 1.2. Additionally, the significance we calculated with is shown in stars: *<0.05, **<0:01, ***<0.001, (scheme based on description by Thomas Kopf).

	LPC 20:0	LPC 19:0	LPC 18:0	LPC 18:1	LPC 16:1	LPC 18:2	LPC 20:3	**LPC 18:3**	LPC 22:4	LPC 20:4	LPC 22:5	LPC 20:5	LPC 22:6
PC 40:7				22:6		22:5	20:4	22:4	18:3	**20:3**	18:2	20:2	18:1
PC 38:6				20:5	22:5	20:4	18:3	**20:3**		18:2	16:1	18:1	**16:0**
PC 40:6			22:6	22:5		22:4	**20:3**		18:2	20:2	18:1		**18:0**
PC 36:5					20:4	18:3		18:2	**14:1**	16:1	**14:0***	**16:0**	
PC 40:5	20:5		22:5	22:4			20:2		18:1		**18:0**	20:0	
PC 42:5	22:5									22:1	20:0	22:0	
PC 34:4*					18:3		**14:1**	16:1	**12:0***	**14:0***			
PC 36:4				18:3	**20:3**	18:2	16:1	18:1	**14:0***	**16:0**			
PC 38:4			20:4	**20:3**		20:2	18:1		**16:0**	**18:0**			
PC 40:4	20:4		22:4					22:1	**18:0**	20:0			
PC 42:4	22:4						22:1	24:1	20:0	22:0			
PC 32:3						**14:1**	**12:0***	**14:0***					
PC 34:3					18:2	16:1	**14:0***	**16:0**					
PC 36:3			18:3	18:2	20:2	18:1	**16:0**	**18:0**					
PC 40:3	**20:3**					22:1	20:0	22:0					
PC 32:2				**14:1**	16:1	**14:0***							
PC 34:2				16:1	18:1	**16:0**							
PC 38:2	18:2		20:2		22:1	20:0							
PC 40:2*	20:2			22:1	24:1	22:0							
PC 30:1				**12:0***	**14:0***								
PC 32:1			**14:1**	**14:0***	**16:0**								
PC 34:1	**14:1**		16:1	**16:0**	**18:0**								
PC 36:1	16:1		18:1	**18:0**	20:0								
PC 38:1	18:1			20:0	22:0								
PC 40:1			22:1	22:0	24:0								
PC 44:1	24:1												
PC 26:0													
PC 28:0			**10:0****										
PC 30:0**	**10:0****		**12:0***										
PC 32:0	**12:0***	13:0	**14:0***										
PC 34:0	**14:0***	**15:0***	**16:0**										
PC 36:0	**16:0**	**17:0**	**18:0**										
PC 40:0	20:0		22:0										
PC 42:0	22:0		24:0										

S 29: Control (8h), increased levels compared to baseline are highlighted in bold (red), decreased levels compared to baseline are highlighted in light grey (blue), and those with no significant changes black. In the middle boxes, those fatty acid species are shown from which the respective PC species could potentially be built with the corresponding LPC species (at the top). PC species are shown on the left. A significant decrease is defined as ≥ 1.2. Additionally, the significance we calculated with is shown in stars: *<0.05, **<0:01, ***<0.001, (scheme based on description by Thomas Kopf).

	LPC 20:0	LPC 19:0	LPC 18:0	LPC 18:1	LPC 16:1	LPC 18:2	LPC 20:3	LPC 18:3	LPC 22:4	LPC 20:4	LPC 22:5	LPC 20:5	LPC 22:6
PC 40:7				22:6		22:5	20:4	22:4	18:3	**20:3**	18:2	**20:2**	18:1
PC 38:6				20:5	22:5	20:4	18:3	**20:3**		18:2	**16:1**	18:1	**16:0**
PC 40:6			22:6	22:5		22:4	**20:3**		18:2	**20:2**	18:1		**18:0***
PC 36:5					20:4	18:3		18:2	**14:1**	**16:1**	**14:0*****	**16:0**	
PC 40:5	20:5		22:5	22:4			**20:2**		18:1		**18:0***	20:0	
PC 42:5	22:5									22:1	20:0	22:0	
PC 34:4					18:3		**14:1**	**16:1**	**12:0*****	**14:0*****			
PC 36:4				18:3	**20:3**	18:2	**16:1**	18:1	**14:0*****	**16:0**			
PC 38:4			20:4	**20:3**		**20:2**	18:1		**16:0**	**18:0***			
PC 40:4	20:4		22:4					22:1	**18:0***	20:0			
PC 42:4	22:4						22:1	24:1	20:0	22:0			
PC 32:3						**14:1**	**12:0*****	**14:0*****					
PC 34:3					18:2	**16:1**	**14:0*****	**16:0**					
PC 36:3			18:3	18:2	**20:2**	18:1	**16:0**	**18:0***					
PC 40:3	**20:3**					22:1	20:0	22:0					
PC 32:2				**14:1**	**16:1**	**14:0*****							
PC 34:2				**16:1**	18:1	**16:0**							
PC 38:2	18:2		**20:2**		22:1	20:0							
PC 40:2	**20:2**			22:1	24:1	22:0							
PC 30:1				**12:0*****	**14:0*****								
PC 32:1			**14:1**	**14:0*****	**16:0**								
PC 34:1	**14:1**		**16:1**	**16:0**	**18:0***								
PC 36:1	**16:1**		18:1	**18:0***	20:0								
PC 38:1	18:1			20:0	22:0								
PC 40:1			22:1	22:0	24:0								
PC 44:1	24:1												
PC 26:0*													
PC 28:0			**10:0*****										
PC 30:0**	**10:0*****		**12:0*****										
PC 32:0	**12:0*****	13:0	**14:0*****										
PC 34:0	**14:0*****	**15:0****	**16:0**										
PC 36:0	**16:0**	**17:0***	**18:0***										
PC 40:0	20:0		22:0										
PC 42:0	22:0		24:0										

S 30: Risk (4h), increased levels compared to baseline are highlighted in bold (red), decreased levels compared to baseline are highlighted in light grey (blue), and those with no significant changes black. In the middle boxes, those fatty acid species are shown from which the respective PC species could potentially be built with the corresponding LPC species (at the top). PC species are shown on the left. A significant decrease is defined as ≥ 1.2. Additionally, the significance we calculated with is shown in stars: : *<0.05, **<0:01, ***<0.001, (scheme based on description by Thomas Kopf).

	LPC 20:0	LPC 19:0	LPC 18:0	LPC 18:1	LPC 16:1	LPC 18:2	LPC 20:3	**LPC 18:3**	LPC 22:4	LPC 20:4	LPC 22:5	LPC 20:5	LPC 22:6
PC 40:7				22:6		22:5	20:4	22:4	18:3	**20:3**	18:2	**20:2**	18:1
PC 38:6				20:5	22:5	20:4	18:3	**20:3**		18:2	16:1	18:1	**16:0**
PC 40:6			22:6	22:5		22:4	**20:3**		18:2	**20:2**	18:1		**18:0***
PC 36:5					20:4	18:3		18:2	**14:1**	16:1	**14:0****	**16:0**	
PC 40:5	20:5		22:5	22:4			**20:2**		18:1		**18:0***	20:0	
PC 42:5	22:5									22:1	20:0	22:0	
PC 34:4*					18:3		**14:1**	16:1	**12:0*****	**14:0****			
PC 36:4				18:3	**20:3**	18:2	16:1	18:1	**14:0****	**16:0**			
PC 38:4			20:4	**20:3**		**20:2**	18:1		**16:0**	**18:0***			
PC 40:4	20:4		22:4					22:1	**18:0***	20:0			
PC 42:4	22:4						22:1	24:1	20:0	22:0			
PC 32:3						**14:1**	**12:0*****	**14:0****					
PC 34:3					18:2	16:1	**14:0****	**16:0**					
PC 36:3			18:3	18:2	**20:2**	18:1	**16:0**	**18:0***					
PC 40:3	**20:3**					22:1	20:0	22:0					
PC 32:2				**14:1**	16:1	**14:0****							
PC 34:2				16:1	18:1	**16:0**							
PC 38:2	18:2		**20:2**		22:1	20:0							
PC 40:2	**20:2**			22:1	24:1	22:0							
PC 30:1				**12:0*****	**14:0****								
PC 32:1			**14:1**	**14:0****	**16:0**								
PC 34:1	**14:1**		16:1	**16:0**	**18:0***								
PC 36:1*	16:1		18:1	**18:0***	20:0								
PC 38:1	18:1			20:0	22:0								
PC 40:1			22:1	22:0	24:0								
PC 44:1	24:1												
PC 26:0													
PC 28:0			**10:0*****										
PC 30:0***	**10:0*****		**12:0*****										
PC 32:0	**12:0*****	13:0	**14:0****										
PC 34:0	**14:0****	**15:0****	**16:0**										
PC 36:0	**16:0**	**17:0***	**18:0***										
PC 40:0	20:0		22:0										
PC 42:0*	22:0		24:0										

S 31: Risk (6h), increased levels compared to baseline are highlighted in bold (red), decreased levels compared to baseline are highlighted in light grey (blue), and those with no significant changes black. In the middle boxes, those fatty acid species are shown from which the respective PC species could potentially be built with the corresponding LPC species (at the top). PC species are shown on the left. A significant decrease is defined as ≥ 1.2. Additionally, the significance we calculated with is shown in stars: : *<0.05, **<0:01, ***<0.001, (scheme based on description by Thomas Kopf).

	LPC 20:0	LPC 19:0	LPC 18:0	LPC 18:1	LPC 16:1	LPC 18:2	LPC 20:3	LPC 18:3	LPC 22:4	LPC 20:4	LPC 22:5	LPC 20:5	LPC 22:6
PC 40:7				22:6		22:5	20:4	22:4	18:3	**20:3**	18:2	**20:2**	18:1
PC 38:6				20:5	22:5	20:4	18:3	**20:3**		18:2	16:1	18:1	**16:0**
PC 40:6			22:6	22:5		22:4	**20:3**		18:2	**20:2**	18:1		18:0
PC 36:5					20:4	18:3		18:2	**14:1**	16:1	**14:0**	**16:0**	
PC 40:5	20:5		22:5	22:4			**20:2**		18:1		18:0	20:0	
PC 42:5	22:5									22:1	20:0	22:0	
PC 34:4*					18:3		**14:1**	16:1	**12:0**	**14:0**			
PC 36:4*				18:3	**20:3**	18:2	16:1	18:1	**14:0**	**16:0**			
PC 38:4*			20:4	**20:3**		**20:2**	18:1		**16:0**	18:0			
PC 40:4	20:4		22:4					22:1	18:0	20:0			
PC 42:4	22:4						22:1	24:1	20:0	22:0			
PC 32:3*						**14:1**	**12:0**	**14:0**					
PC 34:3					18:2	16:1	**14:0**	**16:0**					
PC 36:3			18:3	18:2	**20:2**	18:1	**16:0**	18:0					
PC 40:3	**20:3**					22:1	20:0	22:0					
PC 32:2				**14:1**	16:1	**14:0**							
PC 34:2				16:1	18:1	**16:0**							
PC 38:2	18:2		**20:2**		22:1	20:0							
PC 40:2	**20:2**			22:1	24:1	22:0							
PC 30:1				**12:0**	**14:0**								
PC 32:1*			**14:1**	**14:0**	**16:0**								
PC 34:1*	**14:1**		16:1	**16:0**	18:0								
PC 36:1*	16:1		18:1	18:0	20:0								
PC 38:1	18:1			20:0	22:0								
PC 40:1			22:1	22:0	24:0								
PC 44:1	24:1												
PC 26:0													
PC 28:0			**10:0****										
PC 30:0***	**10:0****		**12:0**										
PC 32:0	**12:0**	13:0	**14:0**										
PC 34:0	**14:0**	**15:0**	**16:0**										
PC 36:0	**16:0**	17:0	18:0										
PC 40:0	20:0		22:0										
PC 42:0	22:0		24:0										

S 32: Risk (8h), increased levels compared to baseline are highlighted in bold (red), decreased levels compared to baseline are highlighted in light grey (blue), and those with no significant changes black. In the middle boxes, those fatty acid species are shown from which the respective PC species could potentially be built with the corresponding LPC species (at the top). PC species are shown on the left. A significant decrease is defined as ≥ 1.2. Additionally, the significance we calculated with is shown in stars: *<0.05, **<0:01, ***<0.001, (scheme based on description by Thomas Kopf).

	LPC 20:0	LPC 19:0	LPC 18:0	**LPC 18:1**	LPC 16:1*	**LPC 18:2**	**LPC 20:3**	**LPC 18:3**	LPC 22:4	LPC 20:4*	LPC 22:5*	LPC 20:5*	**LPC 22:6**
PC 40:7				22:6		22:5	20:4	**22:4**	**18:3**	**20:3**	18:2	**20:2**	18:1
PC 38:6				20:5	22:5	20:4	**18:3**	**20:3**		18:2	16:1	18:1	**16:0**
PC 40:6			22:6	22:5		**22:4**	**20:3**		18:2	**20:2**	18:1		**18:0****
PC 36:5					20:4	**18:3**		18:2	**14:1**	16:1	**14:0*****	**16:0**	
PC 40:5	20:5		22:5	**22:4**			**20:2**		18:1		**18:0****	20:0	
PC 42:5	22:5									22:1	20:0	22:0	
PC 34:4					**18:3**		**14:1**	16:1	**12:0*****	**14:0*****			
PC 36:4				**18:3**	**20:3**	18:2	16:1	18:1	**14:0*****	**16:0**			
PC 38:4			20:4	**20:3**		**20:2**	18:1		**16:0**	**18:0****			
PC 40:4	20:4		**22:4**					22:1	**18:0****	20:0			
PC 42:4	**22:4**						22:1	24:1	20:0	22:0			
PC 32:3						**14:1**	**12:0*****	**14:0*****					
PC 34:3*					18:2	16:1	**14:0*****	**16:0**					
PC 36:3			**18:3**	18:2	**20:2**	18:1	**16:0**	**18:0****					
PC 40:3	**20:3**					22:1	20:0	22:0					
PC 32:2				**14:1**	16:1	**14:0*****							
PC 34:2				16:1	18:1	**16:0**							
PC 38:2*	18:2		**20:2**		22:1	20:0							
PC 40:2	**20:2**			22:1	24:1	22:0							
PC 30:1				**12:0*****	**14:0*****								
PC 32:1			**14:1**	**14:0*****	**16:0**								
PC 34:1	**14:1**		16:1	**16:0**	**18:0****								
PC 36:1	16:1		18:1	**18:0****	20:0								
PC 38:1	18:1			20:0	22:0								
PC 40:1			22:1	22:0	24:0								
PC 44:1	24:1												
PC 26:0													
PC 28:0			**10:0*****										
PC 30:0**	**10:0*****		**12:0*****										
PC 32:0*	**12:0*****	13:0	**14:0*****										
PC 34:0	**14:0*****	**15:0*****	**16:0**										
PC 36:0	**16:0**	**17:0****	**18:0****										
PC 40:0	20:0		22:0										
PC 42:0	22:0		24:0										

S 33: Metabolic syndrome (4h), increased levels compared to baseline are highlighted in bold (red), decreased levels compared to baseline are highlighted in light grey (blue), and those with no significant changes black. In the middle boxes, those fatty acid species are shown from which the respective PC species could potentially be built with the corresponding LPC species (at the top). PC species are shown on the left. A significant decrease is defined as ≥ 1.2. Additionally, the significance we calculated with is shown in stars:: *<0.05, **<0:01, ***<0.001, (scheme based on description by Thomas Kopf).

	LPC 20:0	LPC 19:0	LPC 18:0	LPC 18:1	LPC 16:1	LPC 18:2	LPC 20:3	LPC 18:3	LPC 22:4	LPC 20:4*	LPC 22:5	LPC 20:5	LPC 22:6
PC 40:7				22:6		22:5	20:4	**22:4**	**18:3**	**20:3**	18:2	**20:2**	18:1
PC 38:6				20:5	22:5	20:4	**18:3**	**20:3**		18:2	16:1	18:1	**16:0**
PC 40:6			22:6	22:5		**22:4**	**20:3**		18:2	**20:2**	18:1		**18:0***
PC 36:5					20:4	**18:3**		18:2	**14:1**	16:1	**14:0*****	**16:0**	
PC 40:5	20:5		22:5	**22:4**			**20:2**		18:1		**18:0***	20:0	
PC 42:5	22:5									22:1	20:0	22:0	
PC 34:4					**18:3**		**14:1**	16:1	**12:0*****	**14:0*****			
PC 36:4				**18:3**	**20:3**	18:2	16:1	18:1	**14:0*****	**16:0**			
PC 38:4			20:4	**20:3**		**20:2**	18:1		**16:0**	**18:0***			
PC 40:4	20:4		**22:4**					22:1	**18:0***	20:0			
PC 42:4	**22:4**						22:1	24:1	20:0	22:0			
PC 32:3						**14:1**	**12:0*****	**14:0*****					
PC 34:3					18:2	16:1	**14:0*****	**16:0**					
PC 36:3			**18:3**	18:2	**20:2**	18:1	**16:0**	**18:0***					
PC 40:3	**20:3**					22:1	20:0	22:0					
PC 32:2*				**14:1**	16:1	**14:0*****							
PC 34:2				16:1	18:1	**16:0**							
PC 38:2	18:2		**20:2**		22:1	20:0							
PC 40:2	**20:2**			22:1	24:1	22:0							
PC 30:1				**12:0*****	**14:0*****								
PC 32:1			**14:1**	**14:0*****	**16:0**								
PC 34:1	**14:1**		16:1	**16:0**	**18:0***								
PC 36:1	16:1		18:1	**18:0***	20:0								
PC 38:1	18:1			20:0	22:0								
PC 40:1			22:1	22:0	24:0								
PC 44:1	24:1												
PC 26:0*													
PC 28:0			**10:0*****										
PC 30:0***	**10:0*****		**12:0*****										
PC 32:0*	**12:0*****	13:0	**14:0*****										
PC 34:0	**14:0*****	**15:0*****	**16:0**										
PC 36:0	**16:0**	**17:0****	**18:0***										
PC 40:0	20:0		22:0										
PC 42:0	22:0		24:0										

S 34: Metabolic syndrome (6h), increased levels compared to baseline are highlighted in bold (red), decreased levels compared to baseline are highlighted in light grey (blue), and those with no significant changes black. In the middle boxes, those fatty acid species are shown from which the respective PC species could potentially be built with the corresponding LPC species (at the top). PC species are shown on the left. A significant decrease is defined as ≥ 1.2. Additionally, the significance we calculated with is shown in stars: *<0.05, **<0:01, ***<0.001, (scheme based on description by Thomas Kopf).

	LPC 20:0	LPC 19:0	LPC 18:0	LPC 18:1	LPC 16:1*	LPC 18:2	LPC 20:3	LPC 18:3	LPC 22:4	LPC 20:4	LPC 22:5	LPC 20:5	LPC 22:6
PC 40:7				22:6		22:5	20:4	22:4	**18:3**	**20:3**	18:2	**20:2**	18:1
PC 38:6				20:5	22:5	20:4	**18:3**	**20:3**		18:2	16:1	18:1	**16:0**
PC 40:6			22:6	22:5		22:4	**20:3**		18:2	**20:2**	18:1		**18:0**
PC 36:5					20:4	**18:3**		18:2	**14:1**	16:1	**14:0****	**16:0**	
PC 40:5	20:5		22:5	22:4			**20:2**		18:1		**18:0**	20:0	
PC 42:5	22:5									22:1	20:0	22:0	
PC 34:4					**18:3**		**14:1**	16:1	**12:0*****	**14:0****			
PC 36:4				**18:3**	**20:3**	18:2	16:1	18:1	**14:0****	**16:0**			
PC 38:4			20:4	**20:3**		**20:2**	18:1		**16:0**	**18:0**			
PC 40:4	20:4		22:4					22:1	**18:0**	20:0			
PC 42:4	22:4						22:1	24:1	20:0	22:0			
PC 32:3						**14:1**	**12:0*****	**14:0****					
PC 34:3					18:2	16:1	**14:0****	**16:0**					
PC 36:3			**18:3**	18:2	**20:2**	18:1	**16:0**	**18:0**					
PC 40:3*	**20:3**					22:1	20:0	22:0					
PC 32:2*				**14:1**	16:1	**14:0****							
PC 34:2				16:1	18:1	**16:0**							
PC 38:2	18:2		**20:2**		22:1	20:0							
PC 40:2	**20:2**			22:1	24:1	22:0							
PC 30:1*				**12:0*****	**14:0****								
PC 32:1			**14:1**	**14:0****	**16:0**								
PC 34:1	**14:1**		16:1	**16:0**	**18:0**								
PC 36:1	16:1		18:1	**18:0**	20:0								
PC 38:1	18:1			20:0	22:0								
PC 40:1			22:1	22:0	24:0								
PC 44:1	24:1												
PC 26:0													
PC 28:0			**10:0*****										
PC 30:0***	**10:0*****		**12:0*****										
PC 32:0	**12:0*****	13:0	**14:0****										
PC 34:0	**14:0****	**15:0****	**16:0**										
PC 36:0	**16:0**	**17:0**	**18:0**										
PC 40:0	20:0		22:0										
PC 42:0	22:0		24:0										

S 35: Figure 65: Metabolic syndrome (8h), increased levels compared to baseline are highlighted in bold (red), decreased levels compared to baseline are highlighted in light grey (blue), and those with no significant changes black. In the middle boxes, those fatty acid species are shown from which the respective PC species could potentially be built with the corresponding LPC species (at the top). PC species are shown on the left. A significant decrease is defined as ≥ 1.2. Additionally, the significance we calculated with is shown in stars: *<0.05, **<0:01, ***<0.001, (scheme based on description by Thomas Kopf).

8 References

Reference List

1. Simmons RK, Alberti KG, Gale EA, Colagiuri S, Tuomilehto J, Qiao Q, Ramachandran A, Tajima N, Brajkovich M, I, Ben-Nakhi A, Reaven G, Hama SB, Mendis S, Roglic G (2010) The metabolic syndrome: useful concept or clinical tool? Report of a WHO Expert Consultation. Diabetologia 53: 600-605. 10.1007/s00125-009-1620-4 [doi].

2. World Health Organization (1998) Obesity
preventing and managing the global epidemic : report of a WHO consultation on obesity : Geneva, 3-5 June 1997. Geneva: World Health Organization.

3. Assmann G, Guerra R, Fox G, Cullen P, Schulte H, Willett D, Grundy SM (2007) Harmonizing the definition of the metabolic syndrome: comparison of the criteria of the Adult Treatment Panel III and the International Diabetes Federation in United States American and European populations. Am J Cardiol 99: 541-548. S0002-9149(06)02070-4 [pii];10.1016/j.amjcard.2006.08.045 [doi].

4. Scholze J, Alegria E, Ferri C, Langham S, Stevens W, Jeffries D, Uhl-Hochgraeber K (2010) Epidemiological and economic burden of metabolic syndrome and its consequences in patients with hypertension in Germany, Spain and Italy; a prevalence-based model. BMC Public Health 10: 529. 1471-2458-10-529 [pii];10.1186/1471-2458-10-529 [doi].

5. Hauner H (2013) Stoffwechsel und Pr+ñvention von Adipositas und Diabetes. In: Biofunktionalit+ñt der Lebensmittelinhaltsstoffe. Springer. pp. 123-144.

6. World Health Organization (2000) Obesity: preventing and managing the global epidemic. World Health Organization.

7. Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, Fruchart JC, James WP, Loria CM, Smith SC, Jr. (2009) Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation 120: 1640-1645. CIRCULATIONAHA.109.192644 [pii];10.1161/CIRCULATIONAHA.109.192644 [doi].

8. Ucar E, Huzmeli C, Guven O, Savas N, Gullu M, Asilyoruk S, Kuvandik C, Temizkan A, Kuvandik G (2009) Frequency of metabolic syndrome among hemodialysis

patients according to NCEP-ATP III and IDF definitions. Ren Fail 31: 221-228. 909526800 [pii];10.1080/08860220802669883 [doi].

9. Alberti KG, Zimmet P, Shaw J (2006) Metabolic syndrome--a new world-wide definition. A Consensus Statement from the International Diabetes Federation. Diabet Med 23: 469-480. DME1858 [pii];10.1111/j.1464-5491.2006.01858.x [doi].

10. Hunt KJ, Resendez RG, Williams K, Haffner SM, Stern MP (2004) National Cholesterol Education Program versus World Health Organization metabolic syndrome in relation to all-cause and cardiovascular mortality in the San Antonio Heart Study. Circulation 110: 1251-1257. 10.1161/01.CIR.0000140762.04598.F9 [doi];01.CIR.0000140762.04598.F9 [pii].

11. Eckel RH, Alberti KG, Grundy SM, Zimmet PZ (2010) The metabolic syndrome. Lancet 375: 181-183. S0140-6736(09)61794-3 [pii];10.1016/S0140-6736(09)61794-3 [doi].

12. Monami M, Marchionni N, Masotti G, Mannucci E (2007) IDF and ATP-III definitions of metabolic syndrome in the prediction of all-cause mortality in type 2 diabetic patients. Diabetes Obes Metab 9: 350-353. DOM615 [pii];10.1111/j.1463-1326.2006.00615.x [doi].

13. Lusis AJ, Pajukanta P (2008) A treasure trove for lipoprotein biology. Nat Genet 40: 129-130. ng0208-129 [pii];10.1038/ng0208-129 [doi].

14. Tomkin GH, Owens D (2012) The chylomicron: relationship to atherosclerosis. Int J Vasc Med 2012: 784536. 10.1155/2012/784536 [doi].

15. Wang H, Wang ZH, Kong J, Yang MY, Jiang GH, Wang XP, Zhong M, Zhang Y, Deng JT, Zhang W (2012) Oxidized low-density lipoprotein-dependent platelet-derived microvesicles trigger procoagulant effects and amplify oxidative stress. Mol Med 18: 159-166. molmed.2011.00295 [pii];10.2119/molmed.2011.00295 [doi].

16. Nakajima K, Nakano T, Tokita Y, Nagamine T, Inazu A, Kobayashi J, Mabuchi H, Stanhope KL, Havel PJ, Okazaki M, Ai M, Tanaka A (2011) Postprandial lipoprotein metabolism: VLDL vs chylomicrons. Clin Chim Acta 412: 1306-1318. S0009-8981(11)00212-9 [pii];10.1016/j.cca.2011.04.018 [doi].

17. Toshima S, Hasegawa A, Kurabayashi M, Itabe H, Takano T, Sugano J, Shimamura K, Kimura J, Michishita I, Suzuki T, Nagai R (2000) Circulating oxidized low density lipoprotein levels. A biochemical risk marker for coronary heart disease. Arterioscler Thromb Vasc Biol 20: 2243-2247.

18. Yuasa-Kawase M, Masuda D, Kitazume-Taneike R, Yamashita T, Kawase R, Nakaoka H, Inagaki M, Nakatani K, Tsubakio-Yamamoto K, Ohama T, Toyama-Nakagawa Y, Nishida M, Ishigami M, Saito M, Eto M, Matsuyama A, Komuro I, Yamashita S (2012) Apolipoprotein B-48 to Triglyceride Ratio Is a Novel and Useful Marker

for Detection of Type III Hyperlipidemia after Antihyperlipidemic Intervention. J Atheroscler Thromb . DN/JST.JSTAGE/jat/13052 [pii].

19. Mahley RW, Huang Y (2007) Atherogenic remnant lipoproteins: role for proteoglycans in trapping, transferring, and internalizing. J Clin Invest 117: 94-98. 10.1172/JCI30889 [doi].

20. Burkard M, Huth K (2010) Dyslipoprotein+ñmien. In: Ern+ñhrung und Fasten als Therapie. Springer. pp. 239-253.

21. Gaddi A, Cicero AF, Odoo FO, Poli AA, Paoletti R (2007) Practical guidelines for familial combined hyperlipidemia diagnosis: an up-date. Vasc Health Risk Manag 3: 877-886.

22. Pejic RN, Lee DT (2006) Hypertriglyceridemia. J Am Board Fam Med 19: 310-316. 19/3/310 [pii].

23. Hokanson JE, Austin MA, Zambon A, Brunzell JD (1993) Plasma triglyceride and LDL heterogeneity in familial combined hyperlipidemia. Arterioscler Thromb 13: 427-434.

24. Mateo-Gallego R, Perez-Calahorra S, Cofan M, Baila-Rueda L, Cenarro A, Ros E, Puzo J, Civeira F (2014) Serum lipid responses to weight loss differ between overweight adults with familial hypercholesterolemia and those with familial combined hyperlipidemia. J Nutr 144: 1219-1226. jn.114.191775 [pii];10.3945/jn.114.191775 [doi].

25. Tato F, Vega GL, Tall AR, Grundy SM (1995) Relation between cholesterol ester transfer protein activities and lipoprotein cholesterol in patients with hypercholesterolemia and combined hyperlipidemia. Arterioscler Thromb Vasc Biol 15: 112-120.

26. Wang H, Knaub LA, Jensen DR, Young JD, Hong EG, Ko HJ, Coates AM, Goldberg IJ, de la Houssaye BA, Janssen RC, McCurdy CE, Rahman SM, Soo CC, Shulman GI, Kim JK, Friedman JE, Eckel RH (2009) Skeletal muscle-specific deletion of lipoprotein lipase enhances insulin signaling in skeletal muscle but causes insulin resistance in liver and other tissues. Diabetes 58: 116-124. db07-1839 [pii];10.2337/db07-1839 [doi].

27. Tabbi-Anneni I, Cooksey R, Gunda V, Liu S, Mueller A, Song G, McClain DA, Wang L (2010) Overexpression of nuclear receptor SHP in adipose tissues affects diet-induced obesity and adaptive thermogenesis. Am J Physiol Endocrinol Metab 298: E961-E970. ajpendo.00655.2009 [pii];10.1152/ajpendo.00655.2009 [doi].

28. Wang L, Liu J, Saha P, Huang J, Chan L, Spiegelman B, Moore DD (2005) The orphan nuclear receptor SHP regulates PGC-1alpha expression and energy production in brown adipocytes. Cell Metab 2: 227-238. S1550-4131(05)00260-3 [pii];10.1016/j.cmet.2005.08.010 [doi].

29. Muller G (2011) Let's shift lipid burden--from large to small adipocytes. Eur J Pharmacol 656: 1-4. S0014-2999(11)00093-8 [pii];10.1016/j.ejphar.2011.01.035 [doi].

30. Pereira-Lancha LO, Campos-Ferraz PL, Lancha AH, Jr. (2012) Obesity: considerations about etiology, metabolism, and the use of experimental models. Diabetes Metab Syndr Obes 5: 75-87. 10.2147/DMSO.S25026 [doi];dmso-5-075 [pii].

31. Alkemade A, Yi CX, Pei L, Harakalova M, Swaab DF, la Fleur SE, Fliers E, Kalsbeek A (2012) AgRP and NPY expression in the human hypothalamic infundibular nucleus correlate with body mass index, whereas changes in alphaMSH are related to type 2 diabetes. J Clin Endocrinol Metab 97: E925-E933. jc.2011-3259 [pii];10.1210/jc.2011-3259 [doi].

32. Paquot N, De FJ, Rorive M (2012) [Obesity: a model of complex interactions between genetics and environment]. Rev Med Liege 67: 332-336.

33. Hawkins MA, Stewart JC (2012) Do negative emotional factors have independent associations with excess adiposity? J Psychosom Res 73: 243-250. S0022-3999(12)00201-2 [pii];10.1016/j.jpsychores.2012.07.009 [doi].

34. Lytle LA, Murray DM, Laska MN, Pasch KE, Anderson SE, Farbakhsh K (2012) Examining the Longitudinal Relationship Between Change in Sleep and Obesity Risk in Adolescents. Health Educ Behav . 1090198112451446 [pii];10.1177/1090198112451446 [doi].

35. Racette SB, Deusinger SS, Deusinger RH (2003) Obesity: overview of prevalence, etiology, and treatment. Phys Ther 83: 276-288.

36. Kokkoris P, Pi-Sunyer FX (2003) Obesity and endocrine disease. Endocrinol Metab Clin North Am 32: 895-914.

37. Lafontan M (2014) Adipose tissue and adipocyte dysregulation. Diabetes Metab 40: 16-28. S1262-3636(13)00161-4 [pii];10.1016/j.diabet.2013.08.002 [doi].

38. Milagro FI, Mansego ML, De MC, Martinez JA (2013) Dietary factors, epigenetic modifications and obesity outcomes: progresses and perspectives. Mol Aspects Med 34: 782-812. S0098-2997(12)00082-9 [pii];10.1016/j.mam.2012.06.010 [doi].

39. Karastergiou K, Smith SR, Greenberg AS, Fried SK (2012) Sex differences in human adipose tissues - the biology of pear shape. Biol Sex Differ 3: 13. 2042-6410-3-13 [pii];10.1186/2042-6410-3-13 [doi].

40. Lebovitz HE (2003) The relationship of obesity to the metabolic syndrome. Int J Clin Pract Suppl 18-27.

41. Bray, George A., Bouchard, Claude, and James, W. P. T. (1998) Handbook of obesity. New York: M. Dekker.

42. Singh P, Somers VK, Romero-Corral A, Sert-Kuniyoshi FH, Pusalavidyasagar S, Davison DE, Jensen MD (2012) Effects of weight gain and weight loss on regional fat distribution. Am J Clin Nutr 96: 229-233. ajcn.111.033829 [pii];10.3945/ajcn.111.033829 [doi].

43. Steiner DF, Park SY, Stoy J, Philipson LH, Bell GI (2009) A brief perspective on insulin production. Diabetes Obes Metab 11 Suppl 4: 189-196. DOM1106 [pii];10.1111/j.1463-1326.2009.01106.x [doi].

44. Osterbye T, Jorgensen KH, Fredman P, Tranum-Jensen J, Kaas A, Brange J, Whittingham JL, Buschard K (2001) Sulfatide promotes the folding of proinsulin, preserves insulin crystals, and mediates its monomerization. Glycobiology 11: 473-479.

45. Uchizono Y, Alarcon C, Wicksteed BL, Marsh BJ, Rhodes CJ (2007) The balance between proinsulin biosynthesis and insulin secretion: where can imbalance lead? Diabetes Obes Metab 9 Suppl 2: 56-66. DOM774 [pii];10.1111/j.1463-1326.2007.00774.x [doi].

46. Schmidt RF, Lang F, Thews G, Schmidt RF, Lang F et al. (2005) Physiologie des Menschen
mit Pathophysiologie, version Berlin, Heidelberg: Springer Berlin Heidelberg.

47. Kobayashi H, Mitsui T, Nomura S, Ohno Y, Kadomatsu K, Muramatsu T, Nagasaka T, Mizutani S (2004) Expression of glucose transporter 4 in the human pancreatic islet of Langerhans. Biochem Biophys Res Commun 314: 1121-1125. S0006291X04000233 [pii].

48. Püschel, Gerhard (2011) Taschenlehrbuch Biochemie. Stuttgart: Thieme.

49. Mehnert, Hellmut and Bandmann, Hans Jrgen (1974) Diabetologie in Klinik und Praxis 102 Tabellen. Stuttgart: Thieme.

50. Sugden MC, Holness MJ (2008) Role of nuclear receptors in the modulation of insulin secretion in lipid-induced insulin resistance. Biochem Soc Trans 36: 891-900. BST0360891 [pii];10.1042/BST0360891 [doi].

51. Hotamisligil GS, Shargill NS, Spiegelman BM (1993) Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science 259: 87-91.

52. Gregor MF, Hotamisligil GS (2011) Inflammatory mechanisms in obesity. Annu Rev Immunol 29: 415-445. 10.1146/annurev-immunol-031210-101322 [doi].

53. Yuan M, Konstantopoulos N, Lee J, Hansen L, Li ZW, Karin M, Shoelson SE (2001) Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of Ikkbeta. Science 293: 1673-1677. 10.1126/science.1061620 [doi];293/5535/1673 [pii].

54. Kadowaki T, Yamauchi T (2005) Adiponectin and adiponectin receptors. Endocr Rev 26: 439-451. 26/3/439 [pii];10.1210/er.2005-0005 [doi].

55. Tilg H, Moschen AR (2006) Adipocytokines: mediators linking adipose tissue, inflammation and immunity. Nat Rev Immunol 6: 772-783. nri1937 [pii];10.1038/nri1937 [doi].

56. Yarandi SS, Hebbar G, Sauer CG, Cole CR, Ziegler TR (2011) Diverse roles of leptin in the gastrointestinal tract: modulation of motility, absorption, growth, and inflammation. Nutrition 27: 269-275. S0899-9007(10)00229-7 [pii];10.1016/j.nut.2010.07.004 [doi].

57. Turer AT, Scherer PE (2012) Adiponectin: mechanistic insights and clinical implications. Diabetologia 55: 2319-2326. 10.1007/s00125-012-2598-x [doi].

58. Wang Y, Zhou M, Lam KS, Xu A (2009) Protective roles of adiponectin in obesity-related fatty liver diseases: mechanisms and therapeutic implications. Arq Bras Endocrinol Metabol 53: 201-212. S0004-27302009000200012 [pii].

59. Shibata R, Ouchi N, Takahashi R, Terakura Y, Ohashi K, Ikeda N, Higuchi A, Terasaki H, Kihara S, Murohara T (2012) Omentin as a novel biomarker of metabolic risk factors. Diabetol Metab Syndr 4: 37. 1758-5996-4-37 [pii];10.1186/1758-5996-4-37 [doi].

60. van MG, Voelker DR, Feigenson GW (2008) Membrane lipids: where they are and how they behave. Nat Rev Mol Cell Biol 9: 112-124. nrm2330 [pii];10.1038/nrm2330 [doi].

61. Oresic M, Hanninen VA, Vidal-Puig A (2008) Lipidomics: a new window to biomedical frontiers. Trends Biotechnol 26: 647-652. S0167-7799(08)00240-0 [pii];10.1016/j.tibtech.2008.09.001 [doi].

62. Wallner S, Schmitz G (2011) Plasmalogens the neglected regulatory and scavenging lipid species. Chem Phys Lipids 164: 573-589. S0009-3084(11)00216-7 [pii];10.1016/j.chemphyslip.2011.06.008 [doi].

63. Kotronen A, Velagapudi VR, Yetukuri L, Westerbacka J, Bergholm R, Ekroos K, Makkonen J, Taskinen MR, Oresic M, Yki-Jarvinen H (2009) Serum saturated fatty acids containing triacylglycerols are better markers of insulin resistance than total serum triacylglycerol concentrations. Diabetologia 52: 684-690. 10.1007/s00125-009-1282-2 [doi].

64. Schmitz G, Ecker J (2008) The opposing effects of n-3 and n-6 fatty acids. Prog Lipid Res 47: 147-155. S0163-7827(07)00057-4 [pii];10.1016/j.plipres.2007.12.004 [doi].

65. Barber MN, Risis S, Yang C, Meikle PJ, Staples M, Febbraio MA, Bruce CR (2012) Plasma lysophosphatidylcholine levels are reduced in obesity and type 2 diabetes. PLoS One 7: e41456. 10.1371/journal.pone.0041456 [doi];PONE-D-12-02596 [pii].

66. Sysi-Aho M, Ermolov A, Gopalacharyulu PV, Tripathi A, Seppanen-Laakso T, Maukonen J, Mattila I, Ruohonen ST, Vahatalo L, Yetukuri L, Harkonen T, Lindfors E, Nikkila J, Ilonen J, Simell O, Saarela M, Knip M, Kaski S, Savontaus E, Oresic M (2011) Metabolic regulation in progression to autoimmune diabetes. PLoS Comput Biol 7: e1002257. 10.1371/journal.pcbi.1002257 [doi];PCOMPBIOL-D-11-00535 [pii].

67. Kulkarni P, Getzenberg RH (2009) High-fat diet, obesity and prostate disease: the ATX-LPA axis? Nat Clin Pract Urol 6: 128-131. ncpuro1311 [pii];10.1038/ncpuro1311 [doi].

68. Hoetzl S, Sprong H, van MG (2007) The way we view cellular (glyco)sphingolipids. J Neurochem 103 Suppl 1: 3-13. JNC4721 [pii];10.1111/j.1471-4159.2007.04721.x [doi].

69. Lahiri S, Futerman AH (2007) The metabolism and function of sphingolipids and glycosphingolipids. Cell Mol Life Sci 64: 2270-2284. 10.1007/s00018-007-7076-0 [doi].

70. Tsimikas S, Willerson JT, Ridker PM (2006) C-reactive protein and other emerging blood biomarkers to optimize risk stratification of vulnerable patients. J Am Coll Cardiol 47: C19-C31. S0735-1097(05)03138-4 [pii];10.1016/j.jacc.2005.10.066 [doi].

71. Devaraj S, Swarbrick MM, Singh U, Adams-Huet B, Havel PJ, Jialal I (2008) CRP and adiponectin and its oligomers in the metabolic syndrome: evaluation of new laboratory-based biomarkers. Am J Clin Pathol 129: 815-822. 02973766P4267102 [pii];10.1309/RN84K51B2JJY1Y0B [doi].

72. Ingelsson E, Larson MG, Yin X, Wang TJ, Meigs JB, Lipinska I, Benjamin EJ, Keaney JF, Jr., Vasan RS (2008) Circulating ghrelin, leptin, and soluble leptin receptor concentrations and cardiometabolic risk factors in a community-based sample. J Clin Endocrinol Metab 93: 3149-3157. jc.2008-0207 [pii];10.1210/jc.2008-0207 [doi].

73. Trujillo ME, Scherer PE (2005) Adiponectin--journey from an adipocyte secretory protein to biomarker of the metabolic syndrome. J Intern Med 257: 167-175. JIM1426 [pii];10.1111/j.1365-2796.2004.01426.x [doi].

74. Galletti F, Barbato A, Versiero M, Iacone R, Russo O, Barba G, Siani A, Cappuccio FP, Farinaro E, della VE, Strazzullo P (2007) Circulating leptin levels predict the development of metabolic syndrome in middle-aged men: an 8-year follow-up study. J Hypertens 25: 1671-1677. 10.1097/HJH.0b013e3281afa09e [doi];00004872-200708000-00022 [pii].

75. Li WC, Hsiao KY, Chen IC, Chang YC, Wang SH, Wu KH (2011) Serum leptin is associated with cardiometabolic risk and predicts metabolic syndrome in Taiwanese adults. Cardiovasc Diabetol 10: 36. 1475-2840-10-36 [pii];10.1186/1475-2840-10-36 [doi].

76. Tulk HM, Robinson LE (2009) Modifying the n-6/n-3 polyunsaturated fatty acid ratio of a high-saturated fat challenge does not acutely attenuate postprandial changes in inflammatory markers in men with metabolic syndrome. Metabolism 58: 1709-1716. S0026-0495(09)00240-6 [pii];10.1016/j.metabol.2009.05.031 [doi].

77. Ahmed A, Khan TE, Yasmeen T, Awan S, Islam N (2012) Metabolic syndrome in type 2 diabetes: comparison of WHO, modified ATPIII & IDF criteria. J Pak Med Assoc 62: 569-574.

78. Henkel E, Menschikowski M, Koehler C, Leonhardt W, Hanefeld M (2005) Impact of glucagon response on postprandial hyperglycemia in men with impaired glucose tolerance and type 2 diabetes mellitus. Metabolism 54: 1168-1173. S0026-0495(05)00147-2 [pii];10.1016/j.metabol.2005.03.024 [doi].

79. Hesse M, Meier H, Zeeh B (1997) Spectroscopic methods in organic chemistry.

80. Greaves RF (2012) A guide to harmonisation and standardisation of measurands determined by liquid chromatography - tandem mass spectrometry in routine clinical biochemistry. Clin Biochem Rev 33: 123-132.

81. Marathe GK, Silva AR, de Castro Faria Neto HC, Tjoelker LW, Prescott SM, Zimmerman GA, McIntyre TM (2001) Lysophosphatidylcholine and lyso-PAF display PAF-like activity derived from contaminating phospholipids. J Lipid Res 42: 1430-1437.

82. Watanabe N, Ikeda H, Nakamura K, Ohkawa R, Kume Y, Tomiya T, Tejima K, Nishikawa T, Arai M, Yanase M, Aoki J, Arai H, Omata M, Fujiwara K, Yatomi Y (2007) Plasma lysophosphatidic acid level and serum autotaxin activity are increased in liver injury in rats in relation to its severity. Life Sci 81: 1009-1015. S0024-3205(07)00583-8 [pii];10.1016/j.lfs.2007.08.013 [doi].

83. M++ller, Manfred J. (2007) Ern+ñhrungsmedizinische Praxis: Methoden-Pr+ñvention-Behandlung; mit 219 Tabellen. Springer.

84. Ecker J, Scherer M, Schmitz G, Liebisch G (2012) A rapid GC-MS method for quantification of positional and geometric isomers of fatty acid methyl esters. J Chromatogr B Analyt Technol Biomed Life Sci 897: 98-104. S1570-0232(12)00229-2 [pii];10.1016/j.jchromb.2012.04.015 [doi].

85. Ecker J, Scherer M, Schmitz G, Liebisch G (2012) A rapid GC-MS method for quantification of positional and geometric isomers of fatty acid methyl esters. J Chromatogr B Analyt Technol Biomed Life Sci 897: 98-104. S1570-0232(12)00229-2 [pii];10.1016/j.jchromb.2012.04.015 [doi].

86. BLIGH EG, DYER WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37: 911-917.

87. Liebisch G, Binder M, Schifferer R, Langmann T, Schulz B, Schmitz G (2006) High throughput quantification of cholesterol and cholesteryl ester by electrospray

ionization tandem mass spectrometry (ESI-MS/MS). Biochim Biophys Acta 1761: 121-128. S1388-1981(05)00299-4 [pii];10.1016/j.bbalip.2005.12.007 [doi].

88. Leidl K, Liebisch G, Richter D, Schmitz G (2008) Mass spectrometric analysis of lipid species of human circulating blood cells. Biochim Biophys Acta 1781: 655-664. S1388-1981(08)00138-8 [pii];10.1016/j.bbalip.2008.07.008 [doi].

89. Scherer M, Schmitz G, Liebisch G (2009) High-throughput analysis of sphingosine 1-phosphate, sphinganine 1-phosphate, and lysophosphatidic acid in plasma samples by liquid chromatography-tandem mass spectrometry. Clin Chem 55: 1218-1222. clinchem.2008.113779 [pii];10.1373/clinchem.2008.113779 [doi].

90. Bartlett EM, Lewis DH (1970) Spectrophotometric determination of phosphate esters in the presence and absence of orthophosphate. Anal Biochem 36: 159-167. 0003-2697(70)90343-X [pii].

91. Zemski Berry KA, Murphy RC (2004) Electrospray ionization tandem mass spectrometry of glycerophosphoethanolamine plasmalogen phospholipids. J Am Soc Mass Spectrom 15: 1499-1508. S1044-0305(04)00481-7 [pii];10.1016/j.jasms.2004.07.009 [doi].

92. Sch+ñffler A, Bollheimer C, B++ttner R, Girlich C, Aslanidis C, et al. (2013) Fettgewebsdysfunktion. In: Funktionsdiagnostik in Endokrinologie, Diabetologie und Stoffwechsel. Springer. pp. 175-180.

93. Lao KM (2011) Effects of pro-inflammatory cytokines tumour necorsis factor alpha and interleukin-6 on ghrelin expression in pancreatic AR42J cell line.

94. Gustafsson A, Ajeti V, Ljunggren L (2011) Detection of suPAR in the Saliva of Healthy Young Adults: Comparison with Plasma Levels. Biomark Insights 6: 119-125. 10.4137/BMI.S8326 [doi];bmi-6-2011-119 [pii].

95. Thomas, Lothar (1998) Labor und Diagnose
Indikation und Bewertung von Laborbefunden für die medizinische Diagnostik. Frankfurt/Main: TH-Books Verlagsgesellschaft.

96. Khoo CM, Tan ML, Wu Y, Wai DC, Subramaniam T, Tai ES, Lee J (2013) Prevalence and Control of Hypercholesterolaemia as Defined by NCEP-ATPIII Guidelines and Predictors of LDL-C Goal Attainment in a Multi-Ethnic Asian Population. Ann Acad Med Singapore 42: 379-387.

97. Toth PP (2013) Insulin Resistance, Small LDL Particles, and Risk for Atherosclerotic Disease. Curr Vasc Pharmacol . CVP-EPUB-20130425-2 [pii].

98. Walldius G, Jungner I (2005) Rationale for using apolipoprotein B and apolipoprotein A-I as indicators of cardiac risk and as targets for lipid-lowering therapy. Eur Heart J 26: 210-212. ehi077 [pii];10.1093/eurheartj/ehi077 [doi].

99. Joy T, Hegele RA (2008) Is raising HDL a futile strategy for atheroprotection? Nat Rev Drug Discov 7: 143-155. nrd2489 [pii];10.1038/nrd2489 [doi].

100. Tian L, Fu M (2010) The relationship between high density lipoprotein subclass profile and plasma lipids concentrations. Lipids Health Dis 9: 118. 1476-511X-9-118 [pii];10.1186/1476-511X-9-118 [doi].

101. Simons LA, Dwyer T, Simons J, Bernstein L, Mock P, Poonia NS, Balasubramaniam S, Baron D, Branson J, Morgan J, . (1987) Chylomicrons and chylomicron remnants in coronary artery disease: a case-control study. Atherosclerosis 65: 181-189.

102. Lambert JE, Ryan EA, Thomson AB, Clandinin MT (2013) De novo lipogenesis and cholesterol synthesis in humans with long-standing type 1 diabetes are comparable to non-diabetic individuals. PLoS One 8: e82530. 10.1371/journal.pone.0082530 [doi];PONE-D-13-31381 [pii].

103. Vansaun MN, Mendonsa AM, Lee GD (2013) Hepatocellular proliferation correlates with inflammatory cell and cytokine changes in a murine model of nonalchoholic fatty liver disease. PLoS One 8: e73054. 10.1371/journal.pone.0073054 [doi];PONE-D-13-15753 [pii].

104. Zong G, Ye X, Sun L, Li H, Yu Z, Hu FB, Sun Q, Lin X (2012) Associations of erythrocyte palmitoleic acid with adipokines, inflammatory markers, and the metabolic syndrome in middle-aged and older Chinese. Am J Clin Nutr 96: 970-976. ajcn.112.040204 [pii];10.3945/ajcn.112.040204 [doi].

105. Veret J, Coant N, Berdyshev EV, Skobeleva A, Therville N, Bailbe D, Gorshkova I, Natarajan V, Portha B, Le SH (2011) Ceramide synthase 4 and de novo production of ceramides with specific N-acyl chain lengths are involved in glucolipotoxicity-induced apoptosis of INS-1 beta-cells. Biochem J 438: 177-189. BJ20101386 [pii];10.1042/BJ20101386 [doi].

106. Poudyal H, Brown L (2011) Stearoyl-CoA desaturase: a vital checkpoint in the development and progression of obesity. Endocr Metab Immune Disord Drug Targets 11: 217-231. EMID-DT-ABS-79 [pii].

107. Paton CM, Ntambi JM (2009) Biochemical and physiological function of stearoyl-CoA desaturase. Am J Physiol Endocrinol Metab 297: E28-E37. 90897.2008 [pii];10.1152/ajpendo.90897.2008 [doi].

108. Drobnik W, Liebisch G, Audebert FX, Frohlich D, Gluck T, Vogel P, Rothe G, Schmitz G (2003) Plasma ceramide and lysophosphatidylcholine inversely correlate with mortality in sepsis patients. J Lipid Res 44: 754-761. 10.1194/jlr.M200401-JLR200 [doi];M200401-JLR200 [pii].

109. Shogomori H, Kobayashi T (2008) Lysenin: a sphingomyelin specific pore-forming toxin. Biochim Biophys Acta 1780: 612-618. S0304-4165(07)00205-X [pii];10.1016/j.bbagen.2007.09.001 [doi].

110. Schlitt A, Blankenberg S, Yan D, von GH, Buerke M, Werdan K, Bickel C, Lackner KJ, Meyer J, Rupprecht HJ, Jiang XC (2006) Further evaluation of plasma sphingomyelin levels as a risk factor for coronary artery disease. Nutr Metab (Lond) 3: 5. 1743-7075-3-5 [pii];10.1186/1743-7075-3-5 [doi].

111. Jin J, Zhang X, Lu Z, Perry DM, Li Y, Russo SB, Cowart LA, Hannun YA, Huang Y (2013) Acid sphingomyelinase plays a key role in palmitic acid-amplified inflammatory signaling triggered by lipopolysaccharide at low concentrations in macrophages. Am J Physiol Endocrinol Metab 305: E853-E867. ajpendo.00251.2013 [pii];10.1152/ajpendo.00251.2013 [doi].

112. Maeba R, Maeda T, Kinoshita M, Takao K, Takenaka H, Kusano J, Yoshimura N, Takeoka Y, Yasuda D, Okazaki T, Teramoto T (2007) Plasmalogens in human serum positively correlate with high- density lipoprotein and decrease with aging. J Atheroscler Thromb 14: 12-18. JST.JSTAGE/jat/14.12 [pii].

113. Hennig B, Meerarani P, Ramadass P, Watkins BA, Toborek M (2000) Fatty acid-mediated activation of vascular endothelial cells. Metabolism 49: 1006-1013. S0026-0495(00)97008-2 [pii];10.1053/meta.2000.7736 [doi].

114. Pietilainen KH, Sysi-Aho M, Rissanen A, Seppanen-Laakso T, Yki-Jarvinen H, Kaprio J, Oresic M (2007) Acquired obesity is associated with changes in the serum lipidomic profile independent of genetic effects--a monozygotic twin study. PLoS One 2: e218. 10.1371/journal.pone.0000218 [doi].

115. Eisinger K, Liebisch G, Schmitz G, Aslanidis C, Krautbauer S, Buechler C (2014) Lipidomic analysis of serum from high fat diet induced obese mice. Int J Mol Sci 15: 2991-3002. ijms15022991 [pii];10.3390/ijms15022991 [doi].

116. Rancoule C, Attane C, Gres S, Fournel A, Dusaulcy R, Bertrand C, Vinel C, Treguer K, Prentki M, Valet P, Saulnier-Blache JS (2013) Lysophosphatidic acid impairs glucose homeostasis and inhibits insulin secretion in high-fat diet obese mice. Diabetologia . 10.1007/s00125-013-2891-3 [doi].

117. Ferry G, Tellier E, Try A, Gres S, Naime I, Simon MF, Rodriguez M, Boucher J, Tack I, Gesta S, Chomarat P, Dieu M, Raes M, Galizzi JP, Valet P, Boutin JA, Saulnier-Blache JS (2003) Autotaxin is released from adipocytes, catalyzes lysophosphatidic acid synthesis, and activates preadipocyte proliferation. Up-regulated expression with adipocyte differentiation and obesity. J Biol Chem 278: 18162-18169. 10.1074/jbc.M301158200 [doi];M301158200 [pii].

118. Siess W, Tigyi G (2004) Thrombogenic and atherogenic activities of lysophosphatidic acid. J Cell Biochem 92: 1086-1094. 10.1002/jcb.20108 [doi].

119. Gendaszewska-Darmach E (2008) Lysophosphatidic acids, cyclic phosphatidic acids and autotaxin as promising targets in therapies of cancer and other diseases. Acta Biochim Pol 55: 227-240. 20081628 [pii].

120. Yung YC, Stoddard NC, Chun J (2014) LPA Receptor Signaling: Pharmacology, Physiology, and Pathophysiology. J Lipid Res . jlr.R046458 [pii];10.1194/jlr.R046458 [doi].

121. Sigruener A, Kleber ME, Heimerl S, Liebisch G, Schmitz G, Maerz W (2014) Glycerophospholipid and sphingolipid species and mortality: the Ludwigshafen Risk and Cardiovascular Health (LURIC) study. PLoS One 9: e85724. 10.1371/journal.pone.0085724 [doi];PONE-D-13-36583 [pii].

122. Monteiro R, Azevedo I (2010) Chronic inflammation in obesity and the metabolic syndrome. Mediators Inflamm 2010. 10.1155/2010/289645 [doi].

123. Heraclides A, Jensen TM, Rasmussen SS, Eugen-Olsen J, Haugaard SB, Borch-Johnsen K, Sandbaek A, Lauritzen T, Witte DR (2013) The pro-inflammatory biomarker soluble urokinase plasminogen activator receptor (suPAR) is associated with incident type 2 diabetes among overweight but not obese individuals with impaired glucose regulation: effect modification by smoking and body weight status. Diabetologia . 10.1007/s00125-013-2914-0 [doi].

124. Lyngbaek S, Marott JL, Sehestedt T, Hansen TW, Olsen MH, Andersen O, Linneberg A, Haugaard SB, Eugen-Olsen J, Hansen PR, Jeppesen J (2012) Cardiovascular risk prediction in the general population with use of suPAR, CRP, and Framingham Risk Score. Int J Cardiol . S0167-5273(12)00970-9 [pii];10.1016/j.ijcard.2012.07.018 [doi].

125. Glick NR, Fischer MH (2010) Low DHA and plasmalogens associated with a precise PUFA-rich diet devoid of DHA. Clin Biochem 43: 1305-1308. S0009-9120(10)00381-4 [pii];10.1016/j.clinbiochem.2010.08.021 [doi].

126. Delhanty PJ, Huisman M, Baldeon-Rojas LY, van dB, I, Grefhorst A, Abribat T, Leenen PJ, Themmen AP, van der Lely AJ (2013) Des-acyl ghrelin analogs prevent high-fat-diet-induced dysregulation of glucose homeostasis. FASEB J 27: 1690-1700. fj.12-221143 [pii];10.1096/fj.12-221143 [doi].

127. Kishida K, Funahashi T, Shimomura I (2014) Adiponectin as a routine clinical biomarker. Best Pract Res Clin Endocrinol Metab 28: 119-130. S1521-690X(13)00118-8 [pii];10.1016/j.beem.2013.08.006 [doi].

128. Bergmann K, Sypniewska G (2013) Diabetes as a complication of adipose tissue dysfunction. Is there a role for potential new biomarkers? Clin Chem Lab Med 51: 177-185. 10.1515/cclm-2012-0490 [doi];/j/cclm.2013.51.issue-1/cclm-2012-0490/cclm-2012-0490.xml [pii].

129. Tan BK, Adya R, Randeva HS (2010) Omentin: a novel link between inflammation, diabesity, and cardiovascular disease. Trends Cardiovasc Med 20: 143-148. S1050-1738(10)00159-3 [pii];10.1016/j.tcm.2010.12.002 [doi].

130. Vu A, Sidhom MS, Bredbeck BC, Kosmiski LA, Aquilante CL (2014) Evaluation of the relationship between circulating omentin-1 concentrations and components of the metabolic syndrome in adults without type 2 diabetes or cardiovascular

disease. Diabetol Metab Syndr 6: 4. 1758-5996-6-4 [pii];10.1186/1758-5996-6-4 [doi].

131. Manarin R, Villar SR, Fernandez BR, Gonzalez FB, Deschutter EV, Bonantini AP, Roggero E, Perez AR, Bottasso O (2013) Reciprocal influences between leptin and glucocorticoids during acute Trypanosoma cruzi infection. Med Microbiol Immunol . 10.1007/s00430-013-0294-1 [doi].

132. Vega GL, Grundy SM (2013) Metabolic risk susceptibility in men is partially related to adiponectin/leptin ratio. J Obes 2013: 409679. 10.1155/2013/409679 [doi].

133. Kappelle PJ, Dullaart RP, van Beek AP, Hillege HL, Wolffenbuttel BH (2012) The plasma leptin/adiponectin ratio predicts first cardiovascular event in men: A prospective nested case-control study. Eur J Intern Med . S0953-6205(12)00171-9 [pii];10.1016/j.ejim.2012.06.013 [doi].

134. Granata R, Ghigo E (2013) Products of the ghrelin gene, the pancreatic beta-cell and the adipocyte. Endocr Dev 25: 144-156. 000346306 [pii];10.1159/000346306 [doi].

135. Cheung CK, Wu JC (2013) Role of Ghrelin in the Pathophysiology of Gastrointestinal Disease. Gut Liver 7: 505-512. 10.5009/gnl.2013.7.5.505 [doi].

136. Kovesdy CP, Quarles LD (2013) Fibroblast growth factor-23: what we know, what we don't know, and what we need to know. Nephrol Dial Transplant . gft065 [pii];10.1093/ndt/gft065 [doi].

137. van Oostrom AJ, van DH, Verseyden C, Sniderman AD, Cianflone K, Rabelink TJ, Castro CM (2004) Addition of glucose to an oral fat load reduces postprandial free fatty acids and prevents the postprandial increase in complement component 3. Am J Clin Nutr 79: 510-515.

138. Fielding B (2011) Tracing the fate of dietary fatty acids: metabolic studies of postprandial lipaemia in human subjects. Proc Nutr Soc 70: 342-350. S002966511100084X [pii];10.1017/S002966511100084X [doi].

139. 2002) Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. Circulation 106: 3143-3421.

140. Wojczynski MK, Glasser SP, Oberman A, Kabagambe EK, Hopkins PN, Tsai MY, Straka RJ, Ordovas JM, Arnett DK (2011) High-fat meal effect on LDL, HDL, and VLDL particle size and number in the Genetics of Lipid-Lowering Drugs and Diet Network (GOLDN): an interventional study. Lipids Health Dis 10: 181. 1476-511X-10-181 [pii];10.1186/1476-511X-10-181 [doi].

141. Ramjee V, Sperling LS, Jacobson TA (2011) Non-high-density lipoprotein cholesterol versus apolipoprotein B in cardiovascular risk stratification: do the math. J Am Coll Cardiol 58: 457-463. S0735-1097(11)01706-2 [pii];10.1016/j.jacc.2011.05.009 [doi].

142. Ying X, Qian Y, Jiang Y, Jiang Z, Song Z, Zhao C (2012) Association of the apolipoprotein B/apolipoprotein A-I ratio and low-density lipoprotein cholesterol with insulin resistance in a Chinese population with abdominal obesity. Acta Diabetol . 10.1007/s00592-012-0419-9 [doi].

143. Pitsavos C, Panagiotakos DB, Skoumas J, Papadimitriou L, Stefanadis C (2008) Risk stratification of apolipoprotein B, apolipoprotein A1, and apolipoprotein B/AI ratio on the prevalence of the metabolic syndrome: the ATTICA study. Angiology 59: 335-341. 0003319707307273 [pii];10.1177/0003319707307273 [doi].

144. Coutinho ER, Macedo GM, Campos FS, Bandeira FA (2008) Changes in HDL cholesterol and in the inflammatory markers of atherogenesis after an oral fat load in type-2 diabetic patients and normal individuals. Metab Syndr Relat Disord 6: 153-157. 10.1089/met.2007.0032 [doi].

145. Hartwich J, Leszczynska-Golabek I, Kiec-Wilk B, Siedlecka D, Perez-Martinez P, Marin C, Lopez-Miranda J, Tierney A, Monagle JM, Roche HM, Defoort C, Wolkow P, Dembinska-Kiec A (2010) Lipoprotein profile, plasma ischemia modified albumin and LDL density change in the course of postprandial lipemia. Insights from the LIPGENE study. Scand J Clin Lab Invest 70: 201-208. 10.3109/00365511003663630 [doi].

146. Onat A, Can G, Cicek G, Ayhan E, Dogan Y (2010) Predictive value of serum apolipoprotein B/LDL-cholesterol ratio in cardiometabolic risk: population-based cohort study. Clin Biochem 43: 1381-1386. S0009-9120(10)00390-5 [pii];10.1016/j.clinbiochem.2010.08.027 [doi].

147. Arai H, Kokubo Y, Watanabe M, Sawamura T, Ito Y, Minagawa A, Okamura T, Miyamato Y (2012) Small Dense Low-Density Lipoproteins Cholesterol can Predict Incident Cardiovascular Disease in an Urban Japanese Cohort: The Suita Study. J Atheroscler Thromb . DN/JST.JSTAGE/jat/14936 [pii].

148. Frazier-Wood AC, Glasser S, Garvey WT, Kabagambe EK, Borecki IB, Tiwari HK, Tsai MY, Hopkins PN, Ordovas JM, Arnett DK (2011) A clustering analysis of lipoprotein diameters in the metabolic syndrome. Lipids Health Dis 10: 237. 1476-511X-10-237 [pii];10.1186/1476-511X-10-237 [doi].

149. de Souza JA, Vindis C, Hansel B, Negre-Salvayre A, Therond P, Serrano CV, Jr., Chantepie S, Salvayre R, Bruckert E, Chapman MJ, Kontush A (2008) Metabolic syndrome features small, apolipoprotein A-I-poor, triglyceride-rich HDL3 particles with defective anti-apoptotic activity. Atherosclerosis 197: 84-94. S0021-9150(07)00491-1 [pii];10.1016/j.atherosclerosis.2007.08.009 [doi].

150. Turner RC, Millns H, Neil HA, Stratton IM, Manley SE, Matthews DR, Holman RR (1998) Risk factors for coronary artery disease in non-insulin dependent diabetes mellitus: United Kingdom Prospective Diabetes Study (UKPDS: 23). BMJ 316: 823-828.

151. van DS, Nothlings U, van der Schouw YT, Spijkerman AM, Rutten GE, van der AD, Sluik D, Weikert C, Joost HG, Boeing H, Beulens JW (2011) Non-fasting lipids and risk of cardiovascular disease in patients with diabetes mellitus. Diabetologia 54: 73-77. 10.1007/s00125-010-1945-z [doi].

152. Kolovou GD, Mikhailidis DP, Kovar J, Lairon D, Nordestgaard BG, Ooi TC, Perez-Martinez P, Bilianou H, Anagnostopoulou K, Panotopoulos G (2011) Assessment and clinical relevance of non-fasting and postprandial triglycerides: an expert panel statement. Curr Vasc Pharmacol 9: 258-270. BSP/CVP/E-Pub/0000143 [pii].

153. Pang J, Chan DC, Barrett PH, Watts GF (2012) Postprandial dyslipidaemia and diabetes: mechanistic and therapeutic aspects. Curr Opin Lipidol 23: 303-309. 10.1097/MOL.0b013e328354c790 [doi].

154. Smith D, Watts GF, Dane-Stewart C, Mamo JC (1999) Post-prandial chylomicron response may be predicted by a single measurement of plasma apolipoprotein B48 in the fasting state. Eur J Clin Invest 29: 204-209.

155. Reyes-Soffer G, Holleran S, Karmally W, Ngai CI, Chen NT, Torres M, Ramakrishnan R, Blaner WS, Berglund L, Ginsberg HN, Tuck C (2009) Measures of postprandial lipoproteins are not associated with coronary artery disease in patients with type 2 diabetes mellitus. J Lipid Res 50: 1901-1909. M900092-JLR200 [pii];10.1194/jlr.M900092-JLR200 [doi].

156. Duez H, Lamarche B, Valero R, Pavlic M, Proctor S, Xiao C, Szeto L, Patterson BW, Lewis GF (2008) Both intestinal and hepatic lipoprotein production are stimulated by an acute elevation of plasma free fatty acids in humans. Circulation 117: 2369-2376. CIRCULATIONAHA.107.739888 [pii];10.1161/CIRCULATIONAHA.107.739888 [doi].

157. Kindel T, Lee DM, Tso P (2010) The mechanism of the formation and secretion of chylomicrons. Atheroscler Suppl 11: 11-16. S1567-5688(10)00006-1 [pii];10.1016/j.atherosclerosissup.2010.03.003 [doi].

158. Ng TW, Watts GF, Farvid MS, Chan DC, Barrett PH (2005) Adipocytokines and VLDL metabolism: independent regulatory effects of adiponectin, insulin resistance, and fat compartments on VLDL apolipoprotein B-100 kinetics? Diabetes 54: 795-802. 54/3/795 [pii].

159. Samra JS, Clark ML, Humphreys SM, Macdonald IA, Frayn KN (1996) Regulation of lipid metabolism in adipose tissue during early starvation. Am J Physiol 271: E541-E546.

160. Watts GF, Karpe F (2011) Republished review: Triglycerides and atherogenic dyslipidaemia: extending treatment beyond statins in the high-risk cardiovascular patient. Postgrad Med J 87: 776-782. pgmj.2010.204990rep [pii];10.1136/pgmj.2010.204990rep [doi].

161. Verseyden C, Meijssen S, Castro CM (2002) Postprandial changes of apoB-100 and apoB-48 in TG rich lipoproteins in familial combined hyperlipidemia. J Lipid Res 43: 274-280.

162. Ascaso JF, Real JT, Carmena R (1999) Insulin resistance and familial dyslipidaemias. Diabetes Obes Metab 1: 323-330.

163. Robinson JG (2013) Management of familial hypercholesterolemia: a review of the recommendations from the National Lipid Association Expert Panel on Familial Hypercholesterolemia. J Manag Care Pharm 19: 139-149. 2013(19)2: 139-149 [pii].

164. Liu L, Li Y, Guan C, Li K, Wang C, Feng R, Sun C (2010) Free fatty acid metabolic profile and biomarkers of isolated post-challenge diabetes and type 2 diabetes mellitus based on GC-MS and multivariate statistical analysis. J Chromatogr B Analyt Technol Biomed Life Sci 878: 2817-2825. S1570-0232(10)00533-7 [pii];10.1016/j.jchromb.2010.08.035 [doi].

165. Colas R, Pruneta-Deloche V, Guichardant M, Luquain-Costaz C, Cugnet-Anceau C, Moret M, Vidal H, Moulin P, Lagarde M, Calzada C (2010) Increased lipid peroxidation in LDL from type-2 diabetic patients. Lipids 45: 723-731. 10.1007/s11745-010-3453-9 [doi].

166. Iggman D, Arnlov J, Vessby B, Cederholm T, Sjogren P, Riserus U (2010) Adipose tissue fatty acids and insulin sensitivity in elderly men. Diabetologia 53: 850-857. 10.1007/s00125-010-1669-0 [doi].

167. Yang ZH, Miyahara H, Hatanaka A (2011) Chronic administration of palmitoleic acid reduces insulin resistance and hepatic lipid accumulation in KK-Ay Mice with genetic type 2 diabetes. Lipids Health Dis 10: 120. 1476-511X-10-120 [pii];10.1186/1476-511X-10-120 [doi].

168. Hammad SM, Pierce JS, Soodavar F, Smith KJ, Al Gadban MM, Rembiesa B, Klein RL, Hannun YA, Bielawski J, Bielawska A (2010) Blood sphingolipidomics in healthy humans: impact of sample collection methodology. J Lipid Res 51: 3074-3087. jlr.D008532 [pii];10.1194/jlr.D008532 [doi].

169. Weir JM, Wong G, Barlow CK, Greeve MA, Kowalczyk A, Almasy L, Comuzzie AG, Mahaney MC, Jowett JB, Shaw J, Curran JE, Blangero J, Meikle PJ (2013) Plasma lipid profiling in a large population-based cohort. J Lipid Res 54: 2898-2908. jlr.P035808 [pii];10.1194/jlr.P035808 [doi].

170. Nakamura H, Murayama T (2014) The Role of Sphingolipids in Arachidonic Acid Metabolism. J Pharmacol Sci . DN/JST.JSTAGE/jphs/13R18CP [pii].

171. Demirkan A, Isaacs A, Ugocsai P, Liebisch G, Struchalin M, Rudan I, Wilson JF, Pramstaller PP, Gyllensten U, Campbell H, Schmitz G, Oostra BA, van Duijn CM (2013) Plasma phosphatidylcholine and sphingomyelin concentrations are associated with depression and anxiety symptoms in a Dutch family-based

lipidomics study. J Psychiatr Res 47: 357-362. S0022-3956(12)00341-X [pii];10.1016/j.jpsychires.2012.11.001 [doi].

172. Goto I, Antoku Y, Hayakawa T (1990) Plasma plasmalogens of the phosphoethanolamine type and angiopathy in diabetic patients. Klin Wochenschr 68: 49.

173. Heilbronn LK, Coster AC, Campbell LV, Greenfield JR, Lange K, Christopher MJ, Meikle PJ, Samocha-Bonet D (2013) The effect of short-term overfeeding on serum lipids in healthy humans. Obesity (Silver Spring) . 10.1002/oby.20508 [doi].

174. Pietilainen KH, Rog T, Seppanen-Laakso T, Virtue S, Gopalacharyulu P, Tang J, Rodriguez-Cuenca S, Maciejewski A, Naukkarinen J, Ruskeepaa AL, Niemela PS, Yetukuri L, Tan CY, Velagapudi V, Castillo S, Nygren H, Hyotylainen T, Rissanen A, Kaprio J, Yki-Jarvinen H, Vattulainen I, Vidal-Puig A, Oresic M (2011) Association of lipidome remodeling in the adipocyte membrane with acquired obesity in humans. PLoS Biol 9: e1000623. 10.1371/journal.pbio.1000623 [doi];10-PLBI-RA-8986R3 [pii].

175. Brevik A, Veierod MB, Drevon CA, Andersen LF (2005) Evaluation of the odd fatty acids 15:0 and 17:0 in serum and adipose tissue as markers of intake of milk and dairy fat. Eur J Clin Nutr 59: 1417-1422. 1602256 [pii];10.1038/sj.ejcn.1602256 [doi].

176. Saitoh M, Itoh M, Takashima S, Mizuguchi M, Iwamori M (2009) Phosphatidyl ethanolamine with increased polyunsaturated fatty acids in compensation for plasmalogen defect in the Zellweger syndrome brain. Neurosci Lett 449: 164-167. S0304-3940(08)01539-5 [pii];10.1016/j.neulet.2008.11.004 [doi].

177. Farooqui AA, Horrocks LA (2001) Plasmalogens: workhorse lipids of membranes in normal and injured neurons and glia. Neuroscientist 7: 232-245.

178. Braverman NE, Moser AB (2012) Functions of plasmalogen lipids in health and disease. Biochim Biophys Acta 1822: 1442-1452. S0925-4439(12)00116-0 [pii];10.1016/j.bbadis.2012.05.008 [doi].

179. Catala A (2013) Five Decades with Polyunsaturated Fatty Acids: Chemical Synthesis, Enzymatic Formation, Lipid Peroxidation and Its Biological Effects. J Lipids 2013: 710290. 10.1155/2013/710290 [doi].

180. Nagan N, Zoeller RA (2001) Plasmalogens: biosynthesis and functions. Prog Lipid Res 40: 199-229. S0163-7827(01)00003-0 [pii].

181. Nagan N, Zoeller RA (2001) Plasmalogens: biosynthesis and functions. Prog Lipid Res 40: 199-229. S0163-7827(01)00003-0 [pii].

182. Schiffmann S, Sandner J, Schmidt R, Birod K, Wobst I, Schmidt H, Angioni C, Geisslinger G, Grosch S (2009) The selective COX-2 inhibitor celecoxib modulates sphingolipid synthesis. J Lipid Res 50: 32-40. M800122-JLR200 [pii];10.1194/jlr.M800122-JLR200 [doi].

183. Hartmann D, Lucks J, Fuchs S, Schiffmann S, Schreiber Y, Ferreiros N, Merkens J, Marschalek R, Geisslinger G, Grosch S (2012) Long chain ceramides and very long chain ceramides have opposite effects on human breast and colon cancer cell growth. Int J Biochem Cell Biol 44: 620-628. S1357-2725(11)00366-9 [pii];10.1016/j.biocel.2011.12.019 [doi].

184. Majumdar I, Mastrandrea LD (2012) Serum sphingolipids and inflammatory mediators in adolescents at risk for metabolic syndrome. Endocrine 41: 442-449. 10.1007/s12020-011-9589-4 [doi].

185. Morita Y, Sakaguchi T, Ikegami K, Goto-Inoue N, Hayasaka T, Hang VT, Tanaka H, Harada T, Shibasaki Y, Suzuki A, Fukumoto K, Inaba K, Murakami M, Setou M, Konno H (2013) Lysophosphatidylcholine acyltransferase 1 altered phospholipid composition and regulated hepatoma progression. J Hepatol 59: 292-299. S0168-8278(13)00211-0 [pii];10.1016/j.jhep.2013.02.030 [doi].

186. Lemieux I, Pascot A, Prud'homme D, Almeras N, Bogaty P, Nadeau A, Bergeron J, Despres JP (2001) Elevated C-reactive protein: another component of the atherothrombotic profile of abdominal obesity. Arterioscler Thromb Vasc Biol 21: 961-967.

187. Derosa G, Ferrari I, D'Angelo A, Salvadeo SA, Fogari E, Gravina A, Mereu R, Palumbo I, Maffioli P, Randazzo S, Cicero AF (2009) Oral fat load effects on inflammation and endothelial stress markers in healthy subjects. Heart Vessels 24: 204-210. 10.1007/s00380-008-1109-y [doi].

188. Guerci B, Hadjadj S, Quilliot D, Ziegler O, Drouin P (2000) No acute response of leptin to an oral fat load in obese patients and during circadian rhythm in healthy controls. Eur J Endocrinol 143: 649-655. 1430649 [pii].

189. Hu E, Liang P, Spiegelman BM (1996) AdipoQ is a novel adipose-specific gene dysregulated in obesity. J Biol Chem 271: 10697-10703.

190. Fruebis J, Tsao TS, Javorschi S, Ebbets-Reed D, Erickson MR, Yen FT, Bihain BE, Lodish HF (2001) Proteolytic cleavage product of 30-kDa adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice. Proc Natl Acad Sci U S A 98: 2005-2010. 10.1073/pnas.041591798 [doi];041591798 [pii].

191. Berg AH, Combs TP, Scherer PE (2002) ACRP30/adiponectin: an adipokine regulating glucose and lipid metabolism. Trends Endocrinol Metab 13: 84-89. S1043276001005240 [pii].

192. Annuzzi G, Bozzetto L, Patti L, Santangelo C, Giacco R, Di ML, De NC, Masella R, Riccardi G, Rivellese AA (2010) Type 2 diabetes mellitus is characterized by reduced postprandial adiponectin response: a possible link with diabetic postprandial dyslipidemia. Metabolism 59: 567-574. S0026-0495(09)00366-7 [pii];10.1016/j.metabol.2009.08.020 [doi].

193. Carlson JJ, Turpin AA, Wiebke G, Hunt SC, Adams TD (2009) Pre- and post- prandial appetite hormone levels in normal weight and severely obese women. Nutr Metab (Lond) 6: 32. 1743-7075-6-32 [pii];10.1186/1743-7075-6-32 [doi].

194. Qiao L, Lee B, Kinney B, Yoo HS, Shao J (2011) Energy intake and adiponectin gene expression. Am J Physiol Endocrinol Metab 300: E809-E816. ajpendo.00004.2011 [pii];10.1152/ajpendo.00004.2011 [doi].

195. Li WG, Gavrila D, Liu X, Wang L, Gunnlaugsson S, Stoll LL, McCormick ML, Sigmund CD, Tang C, Weintraub NL (2004) Ghrelin inhibits proinflammatory responses and nuclear factor-kappaB activation in human endothelial cells. Circulation 109: 2221-2226. 10.1161/01.CIR.0000127956.43874.F2 [doi];01.CIR.0000127956.43874.F2 [pii].

196. Cummings DE, Purnell JQ, Frayo RS, Schmidova K, Wisse BE, Weigle DS (2001) A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. Diabetes 50: 1714-1719.

197. Eller LK, Ainslie PN, Poulin MJ, Reimer RA (2008) Differential responses of circulating amylin to high-fat vs. high-carbohydrate meal in healthy men. Clin Endocrinol (Oxf) 68: 890-897. CEN3129 [pii];10.1111/j.1365-2265.2007.03129.x [doi].

198. Delhanty PJ, Neggers SJ, van der Lely AJ (2012) MECHANISMS IN ENDOCRINOLOGY: Ghrelin: the differences between acyl- and des-acyl ghrelin. Eur J Endocrinol 167: 601-608. EJE-12-0456 [pii];10.1530/EJE-12-0456 [doi].

199. le Roux CW, Patterson M, Vincent RP, Hunt C, Ghatei MA, Bloom SR (2005) Postprandial plasma ghrelin is suppressed proportional to meal calorie content in normal-weight but not obese subjects. J Clin Endocrinol Metab 90: 1068-1071. jc.2004-1216 [pii];10.1210/jc.2004-1216 [doi].

200. Kim HH, Lee S, Jeon TY, Son HC, Kim YJ, Sim MS (2004) Post-prandial plasma ghrelin levels in people with different breakfast hours. Eur J Clin Nutr 58: 692-695. 10.1038/sj.ejcn.1601847 [doi];1601847 [pii].

201. Erdmann J, Lippl F, Schusdziarra V (2003) Differential effect of protein and fat on plasma ghrelin levels in man. Regul Pept 116: 101-107. S0167011503001952 [pii].

202. Mirza MA, Alsio J, Hammarstedt A, Erben RG, Michaelsson K, Tivesten A, Marsell R, Orwoll E, Karlsson MK, Ljunggren O, Mellstrom D, Lind L, Ohlsson C, Larsson TE (2011) Circulating fibroblast growth factor-23 is associated with fat mass and dyslipidemia in two independent cohorts of elderly individuals. Arterioscler Thromb Vasc Biol 31: 219-227. ATVBAHA.110.214619 [pii];10.1161/ATVBAHA.110.214619 [doi].

203. Kocelak P, Olszanecka-Glinianowicz M, Chudek J (2012) Fibroblast growth factor 23--structure, function and role in kidney diseases. Adv Clin Exp Med 21: 391-401.

204. Yoda K, Imanishi Y, Yoda M, Mishima T, Ichii M, Yamada S, Mori K, Emoto M, Inaba M (2012) Impaired Response of FGF-23 to Oral Phosphate in Patients with Type 2 Diabetes: A Possible Mechanism of Atherosclerosis. J Clin Endocrinol Metab 97: E2036-E2043. jc.2012-2024 [pii];10.1210/jc.2012-2024 [doi].

Picture sources:

(1): http://www.iaso.org/publications/world-map-obesity

(2): http://www.adipositas-gesellschaft.de/index.php.id=40

(3): http://www.clinicaladvisor.com/a-practical-guide-to-metabolic-syndrome/article/226482/

(4): Müller, G. J Mol Cell Biol (2011) 3 (2): 81-90

(5): http://waswiressen.de/bilder/common/apfel_und _birnentyp_grafik.jpg

(6): http://www.mikrobik.net/page.php?id=273; retrieved 04.12.2012

(7): http://www.hindawi.com/journals/mi/2010/513948/fig5/

(8): http://proteomics.case.edu/proteomics/elisa.html*